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EFFECTS OF ULTRA-VIOLET RADIATION UPON SPORULATION IN MACROSPORIUM AND FUSARIUM[1]

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(WITH PLATE IV AND TWELVE FIGURES)

Introduction

In taxonomic studies of some strains and species of *Macrosporium* and *Alternaria* pathogenic to tomatoes, the difficulty of obtaining spores in artificial culture was encountered. The same problem arose in studies of members of the genus *Fusarium* causing bulb rot of onions. Many strains of *Fusarium* produced microspores in abundance, but failed to produce the macrospores which are necessary for classification of the species. With each genus various methods of obtaining sporulation were tried, such as varying the moisture content of the medium or the kind of medium employed, varying the hydrogen-ion concentration, changing the relative humidity of the air to which the fungus was exposed, varying the temperature, and mutilating the mycelium as recommended by RANDS (8) and KUNKEL (7). Small quantities of spores were obtained at times with these methods, but with no consistency, so that duplication of results could not be relied upon.

[1] Contribution from the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Botany, University of Chicago, cooperating.

[2] The writers are greatly indebted to Professor CHARLES A. SHULL of the Department of Botany for use of the quartz mercury arc, and to Dr. H. B. LEMON and his students in the Department of Physics for use of apparatus and the making of spectrograms.

Field observations have shown that *Macrosporium* and *Alternaria* species affecting tomatoes usually sporulate quite freely on the plants. These fungi produced spores very rarely in pure culture in the laboratory, although good vegetative growth was readily obtained by the usual methods of culturing and under a wide variety of conditions. Since spores were not obtained, a search for the factors favoring sporulation in nature led to a consideration of the effects of light. The fact that sunlight in the laboratory failed to induce sporulation suggested the possibility that it might be the short rays toward the ultra-violet region of the spectrum which were effective. Most of these are excluded by window glass and by the culture vessels. Hence, studies of the effect of ultra-violet radiation upon sporulation in fungi were initiated in November, 1927, at which time a review of the literature failed to reveal any work covering this particular type of reaction to ultra-violet radiation. The results obtained were so favorable (fig. 1) that it was decided to continue the investigations.

Most of the previous investigations of the effects of ultra-violet radiations upon fungi have been concerned with determining the fungicidal action of these rays. BOVIE (1) found that bacteria and spores of various fungi were killed by a 10-minute exposure to wave lengths shorter than 2925 Ångstrom units. With wave lengths longer than this the organisms were not killed by an exposure of 120 minutes. A change of only 25 mμ was sufficient to cause this change in the killing power of light. In 1916 BOVIE (2), working with the Schumann rays (1250–2000 A.u.), reported that exposures which were lethal to hyaline spores were not lethal to such dark-colored spores as those of *Stemphyllium*. He explained the resistance of the dark-colored spores on the ground that the Schumann rays have not sufficient penetrating power to pass through the colored cell walls. Pigmented yeasts are similarly more resistant to ultra-violet than white yeasts, according to the work of TANNER and RYDER (12), who found that yeast was killed by the short rays although it lived longer than bacteria. Their data indicated a direct relation between size of the cell and its resistance to ultra-violet rays. CHAVARRIA and CLARK (4) also cited pigmentation as a defensive mechanism against lethal action. They found that visible and near ultra-violet light

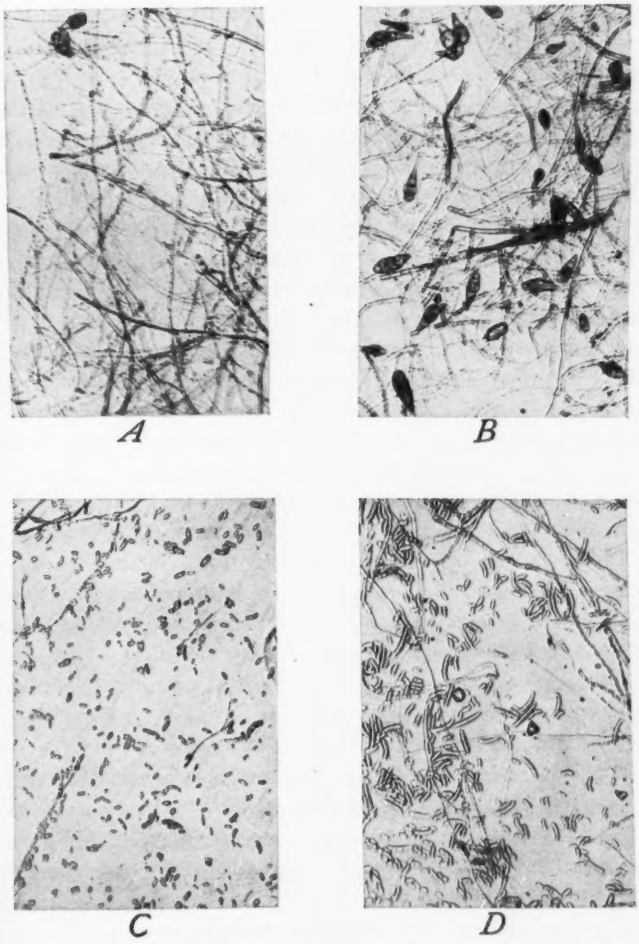

FIG. 1.—*A*, scarcity of spores in control culture of *Macrosporium tomato; B*, spores of same induced by a 15-minute exposure to quartz mercury arc through vitaglass at 40 cm.; *C*, scarcity of macrospores in control culture of *Fusarium cepae; D*, macrospores of *Fusarium cepae* induced by a 15-minute exposure to quartz mercury arc through vitaglass at 40 cm.

together were stimulating in moderate doses to pathogenic fungi of the skin, but had a slightly inhibitory effect in heavy doses in non-pigmented fungi.

Very short exposures are effective in producing marked change in some organisms. An irradiation of two or three minutes at a distance of 15 cm. from a mercury arc is sufficient to cause cytolysis in zoospores of *Blepharospora* placed in a thin film of water (DUFRENOY 5). Even shorter exposures are sufficient to kill the spores of *Fusarium echinosporum* and *F. fuliginosporum*, according to SIBILIA (9).

In recent studies of the fungicidal action of ultra-violet radiations, FULTON and COBLENTZ (6) found that the spores of the twenty-seven miscellaneous species of fungi under observation were killed in most instances by a comparatively short exposure to ultra-violet radiations. The different wave-length components showed different killing power, which increased as the wave length decreased, the lethal action beginning at about 365 mμ as an upper limit.

In addition to a lethal effect on the aerial mycelium and spores of *Glomerella cingulata*, STEVENS (10, 11) reported a stimulation of production of acervuli and perithecia when the fungus was exposed to ultra-violet rays. He found that direct radiation from a quartz mercury arc for periods of 0.25 to 4 seconds resulted in the production of surface perithecia in agar colonies, and that longer dosage (5 seconds to 2 minutes) resulted in buried perithecia. Colonies as young as two days produced perithecia, but not so many as were produced on colonies 4 days old. The efficient rays of the ultra-violet were found to be throughout the region with wave lengths shorter than 313 mμ. *Coniothyrium* sp. was induced to produce pycnidia earlier than normal by an irradiation of 10 seconds.

Investigation

The present work is not concerned with the lethal effects of the ultra-violet rays but is confined to a study of the production of spores as a result of exposure. In all experiments a Cooper-Hewitt quartz mercury arc lamp was used, operated on direct current at 4 amperes through resistance from a 110 volt line. Exposures were made at a distance of 40 cm. from the arc unless otherwise stated.

In the first experiments lids for petri dishes were made by molding

paraffin rings on squares of vitaglass.[3] These were sterilized with alcohol and allowed to dry before substituting them for the regular petri dish lid, previous to exposure. Plantings of the fungi were made on potato-dextrose agar, and they were allowed to grow until the colonies attained a diameter of approximately 2.5 cm. before irradiation was begun. In all experiments several plates were used for each exposure and the average reading of many mounts obtained. The results were recorded in terms of percentage of macrospores in *Fusarium*, and in number of spores per low power field of the microscope in *Macrosporium*. Controls were held for an equal length of time in the diffused light of the laboratory at room temperature. In preliminary experiments exposures were made for varying periods of time from one minute up to one hour. Irradiations were given for six successive days and spore counts were made on the seventh day.

Although sufficient preliminary tests have been made with several strains of *Macrosporium* and species of *Fusarium* to show that the treatment may have quite wide application, the present discussion is confined to the work with the fungus causing Nailhead Spot of tomatoes, *Macrosporium tomato* CKe.[4] and *Fusarium cepae* Hanz emend Link & Bailey. Detailed studies were made of these two fungi at this time.

The results of a typical experiment with *Macrosporium tomato* are shown in fig. 2. It will be noted that even with the 1-minute exposure at 60 cm. under vitaglass the average number of spores per low power field was about twelve times that of the control. The number of spores increased with the increase in length of exposure, the 60-minute plates having forty-four times as many spores as the controls. In fig. 3 the results of a similar experiment with *Fusarium cepae* show that the percentage of macrospores increased with the length of exposure. Counts of many fields showed that an exposure of one minute gave an average of 12 per cent macrospores as compared with the 5 per cent in the control, while an exposure of one hour increased the macrospores to 65 per cent. Although the control plates showed no exposed pionnotes or sporodochia, but a sea-shell

[3] Vitaglass furnished by courtesy of Vitaglass Corporation, New York City.

[4] According to ROSENBAUM (Phytopath. 10:9–22. 1920). The writers find this fungus produces spores in long chains in a humid atmosphere.

pink[5] to pale salmon-colored fluffy mycelium, in plates exposed five minutes there was an exposed pionnotes over 2 cm. in diameter with small salmon-colored sporodochia under the mycelium beyond the pionnotes. The cultures exposed one hour showed no aerial mycelium but salmon-colored pionnotes covered the plate. Irradiation through

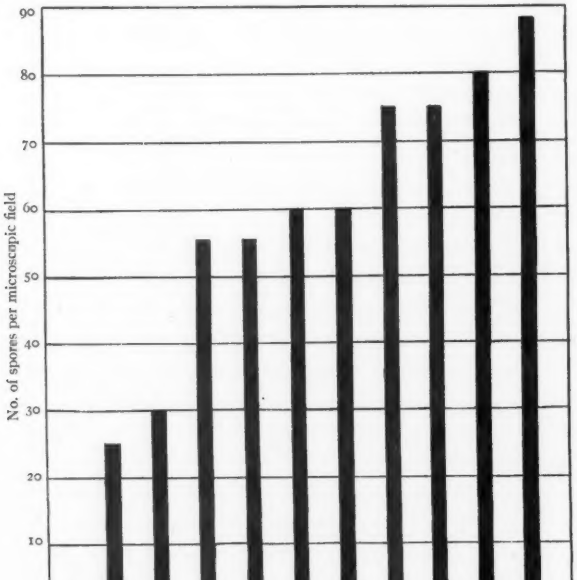

FIG. 2.—Effect on spore production of exposing *Macrosporium tomato* cultures at 60 cm. from quartz mercury arc through vitaglass for various periods of time on six successive days.

vitaglass produced a slight diminution in growth rate (figs. 13 *A*, *C*; 14). The controls showed an average daily increase in diameter of 6 mm., whereas the 1-minute plates showed an average increase of 5.25 mm., the 5-minute plates 4.75 mm., the 10- and 20-minute plates 4.5 mm., and the hour plates 4.3 mm. No abnormal or disrupted spores occurred.

[5] RIDGWAY, R., Color standards and color nomenclature. Washington, D.C. 1912.

Check experiments were run to determine whether heat produced by the arc might be the factor responsible for inducing spore production. Plates were inoculated as before and, except for one hour a day, were kept with control cultures in diffuse light in the laboratory, at approximately 22° C. Exposures were for one hour at 60 cm. from the arc on six successive days. The results obtained from these experiments are given in table I, which clearly shows that temperature is not the important factor in determining sporulation.

Change in the medium due to irradiation was eliminated as a causal factor of increased sporulation by the following experiment. Duplicate sets of plates of potato dextrose agar were poured and covered with vitaglass. One set of these was irradiated before inoculation with the fungi, two plates for 15 minutes, two for 30 minutes, and two for one hour each day. After the third irradiation these plates and the duplicate unirradiated plates were all inoculated with *Macrosporium*. The test was also duplicated for *Fusarium*. After the

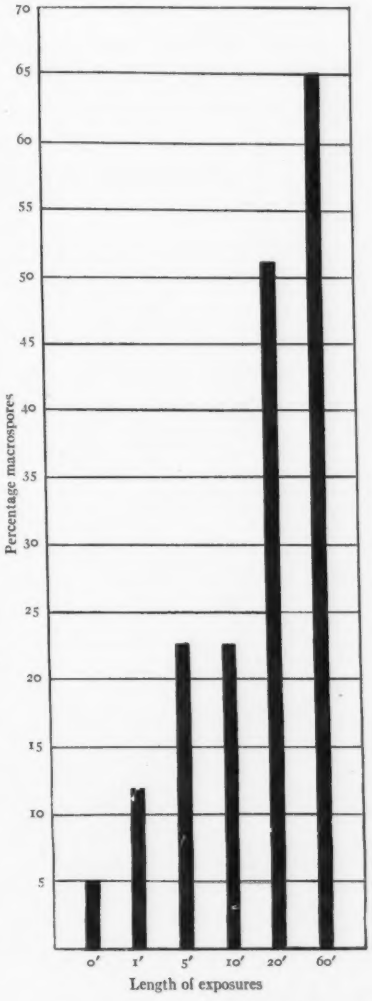

FIG. 3.—Effect on spore production of exposing *Fusarium cepae* cultures at 60 cm. from quartz mercury arc through vitaglass for various periods of time on six successive days.

fungi had been allowed to grow for one day, the unirradiated plates were exposed for the same periods (15, 30, and 60 minutes) to the mercury arc on three successive days. At the end of this time all plates were examined. Those with the medium irradiated before inoculation showed no increase over control plates kept in diffuse light, but those exposed after inoculation all showed a great increase in spore production.

TABLE I

COMPARATIVE EFFECT OF ULTRA-VIOLET RADIATION AND OF TEMPERATURE ON GROWTH AND SPORULATION OF MACROSPORIUM TOMATO AND FUSARIUM CEPAE

Treatment	Temperature in °C. during exposure						Results obtained 5/30			
							Fusarium		Macrosporium	
	5/24	5/25	5/26	5/27	5/28	5/29	Average daily growth in (mm.)	Macrospores (per cent)	Average daily growth (mm.)	Spores
Exposed under vita-glass covers 1 hour daily (transmission to 2650 A.u.)	40.0	32.5	28.0	29.0	30.5	30.0	4.0	50	3.2	Abundant
Exposed under Jena petri dish lids 1 hour daily (transmission to 2800 A.u.)	40.0	32.5	28.0	29.0	30.5	30.0	4.5	40	3.3	Abundant
Exposed under ordinary glass covers 1 hour daily (transmission to 3020 A.u)	40.0	32.5	28.0	29.0	30.5	30.0	4.8	10	3.3	Many spores (less than above)
Petri dish covered with black paper, placed under arc 1 hour daily	35.5	31.0	26.0	24.0	27.0	26.0	5.5	1	3.3	Few
Petri dish placed in incubator 1 hour daily	40.0	35.0	29.0	28.0	30.0	33.0	3.0	3	2.0	Rare
Petri dish kept in diffuse light of laboratory	25.5	25.0	23.0	20.0	20.0	22.0	5.0	2	3.3	Very few

The work thus far showed that the rays produced by a quartz mercury arc are effective in inducing sporulation. Since many radiations in the visible spectrum above 4000 A.u. were also produced,

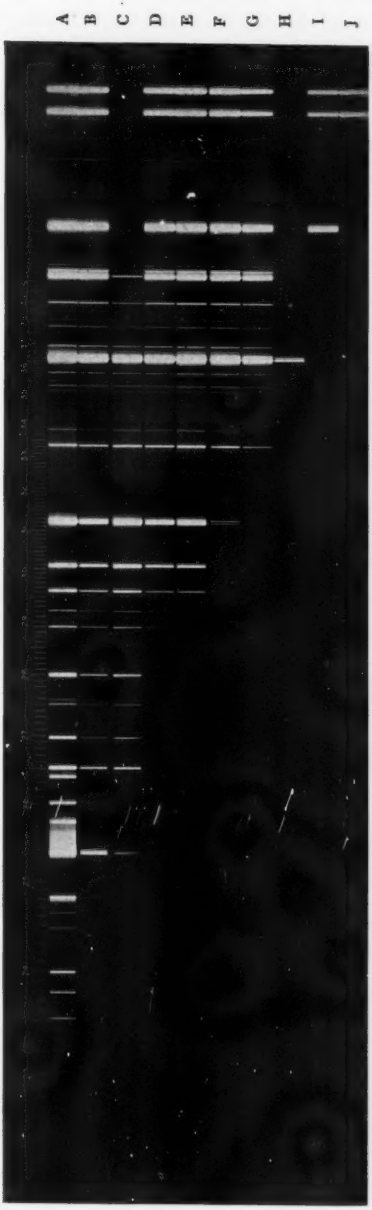

FIG. 4.—Spectrograms of quartz mercury arc direct and through various filters: *A*, direct arc (2230–6690 A.u.); *B*, Corning Corex A (980A), (2300–6690 A.u.); *C*, Corex Red Purple (986A), (2535–4340 A.u.); *D*, vitaglass (2650–6690 A.u.); *E*, petri dish (2800–6690 A.u.); *F*, glass no. 1 (3020–6690 A.u.); *G*, glass no. 6 (3120–6690 A.u.); *H*, Corning (G586AW), (3334–3690 A.u.); *I*, Noviol A (4340–6690 A.u.); *J*, Noviol C (4880–6690 A.u.). Some faint lines showing lowest limits of transmission are lost in reproduction.

it seemed desirable to try to determine the effective region by the use of filters. Consequently a number of filters, each limited in its transmission to definite regions of the spectrum, were procured with the idea of forming a series which would delimit the various portions of the spectrum. The spectrograms of these filters together with that of the mercury arc are shown in fig. 4.

Numerous experiments were then conducted in which these filters as well as the direct lamp were used to test the effectiveness of the

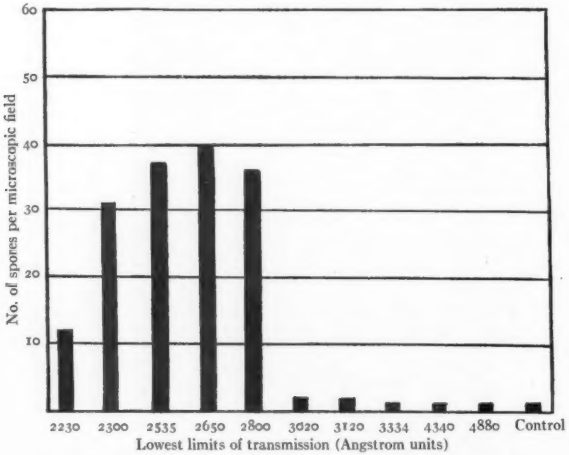

FIG. 5.—Composite graph showing effect on spore production of all experiments in which *Macrosporium tomato* was exposed through various filters at 40 cm. from the quartz mercury arc for 15 minutes.

various radiations upon sporulation of the fungi under consideration. The results of typical experiments with the tomato *Macrosporium* are shown in fig. 5. The most pronounced results were obtained by use of filters whose lower limits of transmission lie between 2535 and 2800 Ångstrom units. Optimum sporulation occurred with the filter whose lower limit was 2650 A.u. It will be noted that direct exposure to the arc caused a marked increase in spore production as compared with the controls, but less increase than that produced with any filter that eliminated the shortest rays but transmitted those between 2300 and 2800 A.u. The inhibiting effect of the short

rays from the direct arc is shown in the appressed, poorly developed mycelium (fig. 15 *B*), as well as in the amount of spores produced. There was practically no influence on the rate of growth when filters were used (fig. 16 *A*, *B*). The fungus on each of these plates grew at approximately the same rate as the controls, which showed an average daily increase of 3.5 mm. in diameter. The irradiated plates

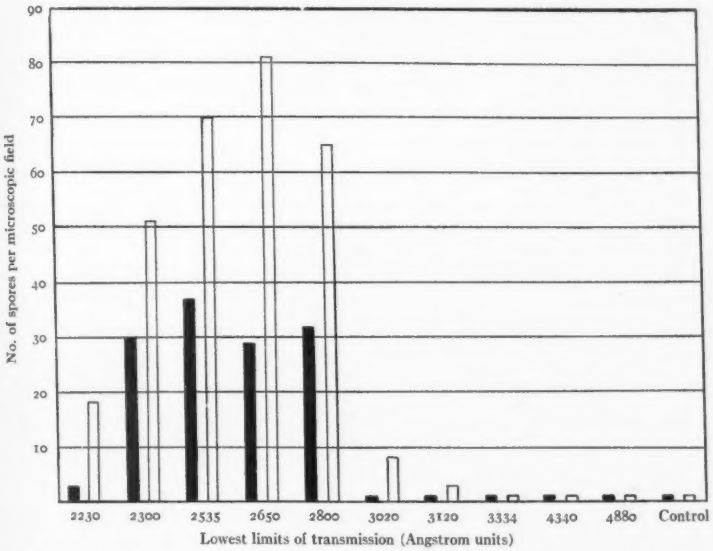

FIG. 6.—Effect on spore production by exposing cultures of *Macrosporium tomato* to quartz mercury arc at 40 cm. through various filters for 15 minutes on two and three successive days: heavy line, 2-day exposure; light line, 3-day exposure.

showed a grayish green mycelial growth (fig. 15 *A*), whereas the controls were grayish brown and tended to be slightly more flocculent (fig. 15 *C*).

The effects of varying the number of exposures are shown in fig. 6. Plates exposed two successive days for 15-minute periods showed upon examination on the third day a very decided increase in spore production, varying from three times as many spores as the control in the case of exposure to the direct arc, to thirty-seven times as many with the use of the Corex Red Purple filter which transmits

down to 2535 A.u. Readings made after three daily exposures of 15 minutes each showed a great increase in all instances where filters which transmit between 2300 and 2800 A.u. were used. There was no change in the control. The maximum stimulation (eighty-one times as many spores as control) was obtained by use of the vitaglass filter which did not transmit below 2650 A.u. In all experiments there was a very sharp decline in the number of spores produced with filters which transmit nothing below 3020 A.u. There was a slight increase in spore production when cultures were irradiated through filters which transmit down to 3120 A.u., but, as shown in the graph, no filters whose lower limits of transmission are above this line of the spectrum were effective in inducing spore production in this species of *Macrosporium*.

Fig. 7 shows the results of exposing *F. cepae* through the various filters, for 15 minutes each on one, two, and three days. This graph is an average of four experiments. The highest percentage of macrospores was obtained by using the vitaglass filter which transmits to 2650 A.u. An increase in the number of exposures increased the percentage of macrospores. The percentage of macrospores produced under vitaglass in three days, as shown in this graph, is 8.3. In some other experiments, however, as high as 17.5 per cent macrospores was produced in three exposures of 15 minutes each (fig. 10). In all experiments, however, the numbers of macrospores produced under these filters bore the same relation to each other as that indicated in fig. 7, with the peak at 2650 A.u. In experiments with exposures of an hour the results followed the same curve.

No increase in production of macrospores occurred with filters transmitting no lower than 3334 A.u. The open arc and the two filters transmitting below 2650 A.u. caused stimulation but less than that produced by exposure through vitaglass. That this decrease in spore production is due to the presence of the shorter lethal rays in these three cases is upheld by microscopic examination. Many of the spores were plasmolyzed, and some, especially those exposed to the open arc, were disrupted. The contents of the plasmolyzed cells of conidia and mycelium were broken up into small globular masses. SIBILIA (9) reports a similar condition for *F. fuliginosporum* and *F. echinosporum*. In the case of disrupted spores these globules were

free and showed as loose aggregations among the hyphae. A marked decrease in growth rate and stunting of the colony (fig. 13 *B*) ac-

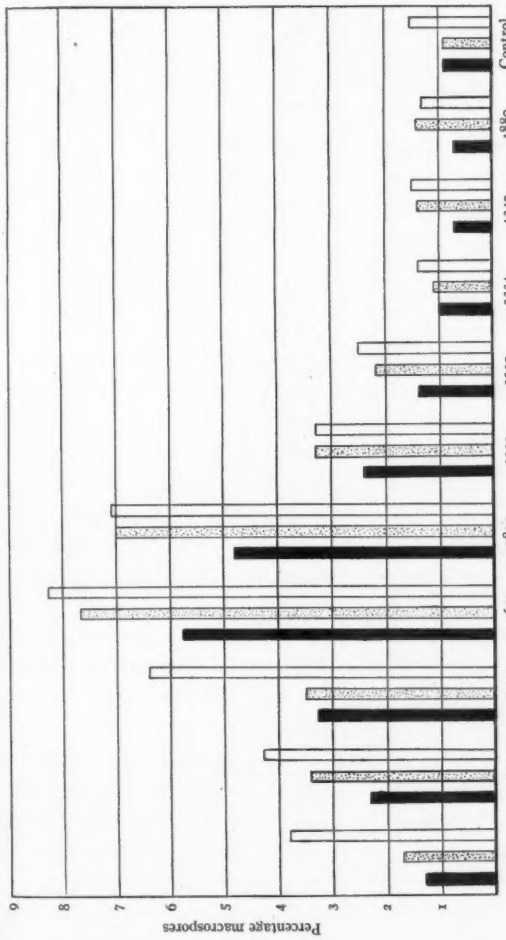

FIG. 7.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through various filters for 15 minutes on one, two, and three days: heavy lines, 1 day 15 min.; dotted lines, 2 days 15 min. each; light lines, 3 days 15 min. each.

companied the lethal effect on the spores after exposure to the direct arc. The fungus was not entirely killed by three successive exposures of one hour, however, for it continued to grow and produce

spores. Of the spores present 24 hours after the third exposure, 18 per cent germinated in three days in distilled water.

The three filters whose lower limits of transmission lie between 2800 and 3120 A.u. also showed an increase in the percentage of macrospores over the control, the percentage varying inversely with the lower limit of transmission. The smaller increase in percentage of macrospores obtained when these filters were used as compared with that occurring with the use of vitaglass is not, however, due to lethal effects. Microscopic examination of spores and mycelium produced under these conditions revealed only normal cells (fig. 1*D*). Irradiation through these filters produced no consistent colony stunting, but the growth rate was approximately the same as in the control. Since there was no microscopic evidence of lethal effects, the slight decrease in growth rate (fig. 14) on exposure through these filters and through vitaglass might seem to be the result of the metabolic changes accompanying the assumption of sporulation rather than of direct inhibition caused by irradiation. In the case of *Macrosporium* no decrease in growth rate occurred with filters which transmitted no lower than 2800 A.u. (table I), and the decrease with vitaglass was so slight as to be negligible (fig. 16), although marked increase in sporulation occurred. In neither fungus was inhibition in growth rate, due to increased temperature, accompanied by sporulation (table I).

Since by the use of the vitaglass filter a maximum number of macrospores was obtained, this filter was used in experiments to determine the minimum exposure necessary for inducing an increase in the number of macrospores. Fig. 8 shows the results of exposures for various lengths of time, from one-hundredth of a second to one hour. A photographic shutter was used in making all exposures shorter than one minute. As shown in fig. 8, there was no definite increase in the percentage of macrospores produced with exposures less than 30 seconds on three successive days, although an exposure of one second gave a marked increase in the total number of microspores and in the percentage of septate microspores. The percentage of macrospores produced varied directly with the length and number of exposures. (In the particular experiment shown in this graph there was a slight irregularity in the 15-minute exposure for one day.)

It will be noted, however, that three successive short exposures are more effective than a single exposure of a duration equal to, or even

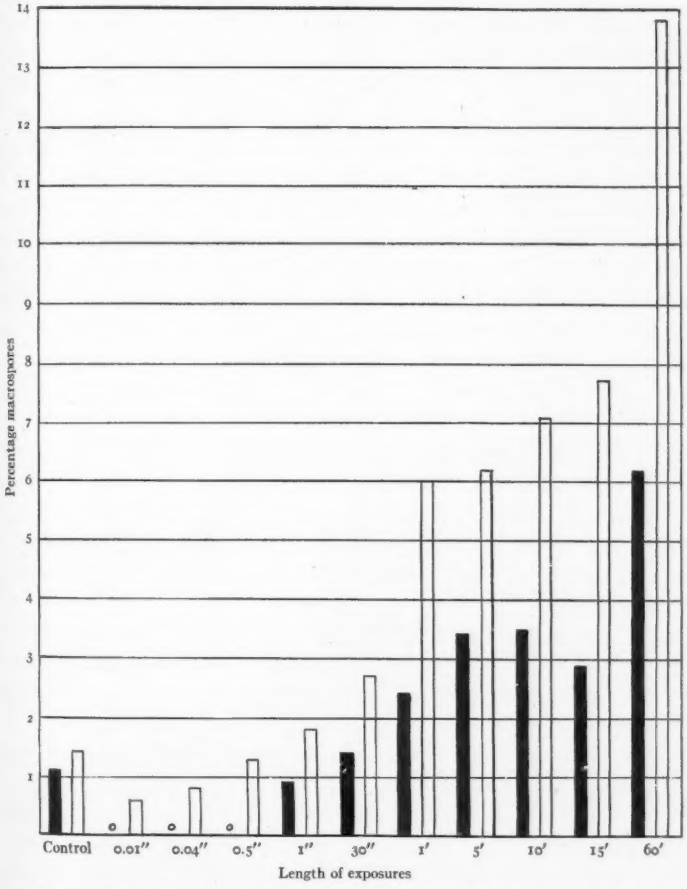

FIG. 8.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through vitaglass for various periods of time on one day and for three successive days: heavy lines, 1-day exposure; light lines, 3-day exposure.

somewhat greater than their total. For example, a 1-minute exposure on each of three days induced the production of more spores

than five minutes on one day; and ten minutes on each of three days was more effective than one hour on one day. In a similar experiment with the filter transmitting down to 2300 A.u., a single exposure of 30 seconds was sufficient to produce an increase in percentage of macrospores.

In experiments with *Macrosporium* in which a Corex A filter transmitting down to 2300 A.u. was used, a slight increase in spore production was evident with a single exposure of one-tenth of a second. With the use of vitaglass, three successive daily exposures of one second produced a slight increase, while with a 30-second exposure a decided increase occurred. The more favorable results obtained with *Macrosporium* on exposures to the shorter wave lengths, as compared with *Fusarium*, is in accordance with the work of others, who found that pigmentation was a protection against the lethal action of these rays. Microscopic examination of cultures exposed for longer periods of time to the direct arc and to the rays transmitted by the Corex A (2300 A.u.) and Corex Red Purple (2535 A.u.) filters showed no disrupting of spores such as appeared in *Fusarium* under similar exposures.

Since increased spore production occurred with all the filters transmitting as low as 3120 A.u., but the amount of increase varied with the different filters, the question arose as to whether the results obtained were due to differences in the wave length or to intensity. As a preliminary attempt to determine what part intensity played in the stimulation of spore production, petri dishes with vitaglass lids were inoculated with *Macrosporium* and exposed for fifteen minutes, at varying distances from the arc (10, 20, 40, 60, 80, and 100 cm. respectively). Readings were made after one exposure and again two days later after three exposures. The results, as recorded in fig. 9, show that the maximum number of spores occurred in the plates exposed at a distance 20 cm. from the arc. The plates exposed at 40 and 60 cm. were practically the same in spore production, but a noticeable decrease in the number of spores was evident in the plates at 80 cm. and 100 cm. The cultures irradiated at 10 cm. gave about the same results as the two more distant ones. Perhaps at this close proximity to the arc there was some inhibitive effect due to the short waves transmitted by the vitaglass. The intensity at 80 cm., where

the big drop in spore production occurred, would be one-sixteenth (four-sixty-fourths), of that at 20 cm., where maximum production occurred. With this decrease in intensity is correlated a decrease to approximately one-third the number of spores, while in the case of the 40 and 60 cm. plates, intensity one-fourth and one-ninth as great, respectively, is correlated in both cases with a spore production two-thirds as great as the 20 cm. plates. Intensity is evidently a factor in the limitation of the number of spores produced.

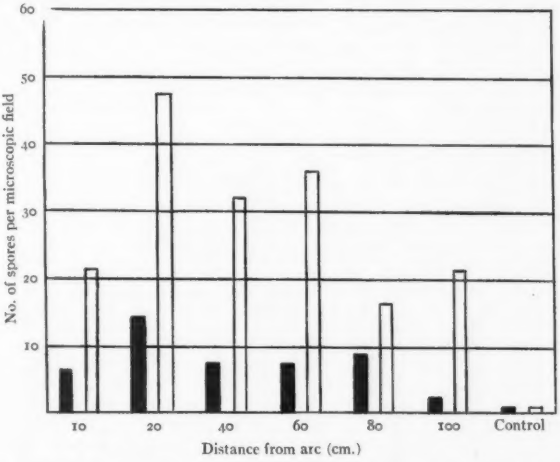

FIG. 9.—Effect on spore production by exposing cultures of *Macrosporium tomato* to quartz mercury arc through vitaglass for 15 minutes at varying distances on one day and for three successive days: heavy lines, 1-day exposure; light lines, 3-day exposure.

Cultures of *Fusarium* exposed at 10, 20, 40, and 60 cm. from the arc gave practically identical results. The 3-day exposures of fifteen minutes each at 40 cm. showed a slightly greater increase in percentage of macrospores (fig. 10); however, 1-hour exposures on three successive days at 40 and 60 cm. slightly reversed the results, so that it would seem that this slight variation is negligible. As in the case of *Macrosporium*, there was a definite decrease in the percentage of spores produced, when cultures were exposed at 80 and 100 cm., these cultures producing less than half as many spores as those exposed nearer the arc. Cutting down the intensity to one-thirty-sixth

was not enough to decrease the production of spores, but reducing it to one-sixty-fourth resulted in a decrease of approximately 50 per cent.

Since there is no adequate method of measuring the relative intensity of ultra-violet radiation passing through the various filters, an attempt was made to answer in part the question as to whether intensity or wave length was responsible for the variation in spore production under the various filters, by making longer exposures in

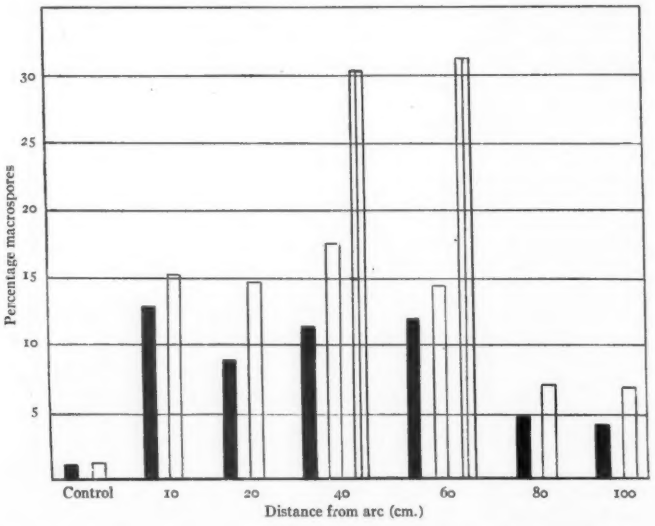

FIG. 10.—Effects on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc through vitaglass at various distances on one day for 15 minutes and on three days for 15 minutes and 1-hour periods: heavy lines, 1 day 15 min.; light line, 3 days 15 min. each; perpendicular line, 3 days 1 hour each.

the case of those filters which gave poor results with short exposure. Consequently, a series of exposures were made (fig. 11), the longest irradiation being six hours on each of three successive days. Exposures through Corning filter 586 A.W. (transmitting to 3334 A.u.) totaling eighteen hours did not induce sporulation, but the longer exposure did produce better results with the glass filters transmitting to 3020 and 3120 A.u. respectively. It will be noted, however, that

multiplying the exposure under these glass filters by 360 only doubled the spore production of a 1-minute vitaglass exposure. A 15-minute exposure under vitaglass produced twice as high a percentage of macrospores as was present under either of the other glasses following six hours' exposure, and even three successive daily exposures totaling eighteen hours, or seventy-two times as long an exposure, failed to produce as many spores as the vitaglass culture. In the spectrogram (fig. 4 *F*) the band at 3120 is prominent in the

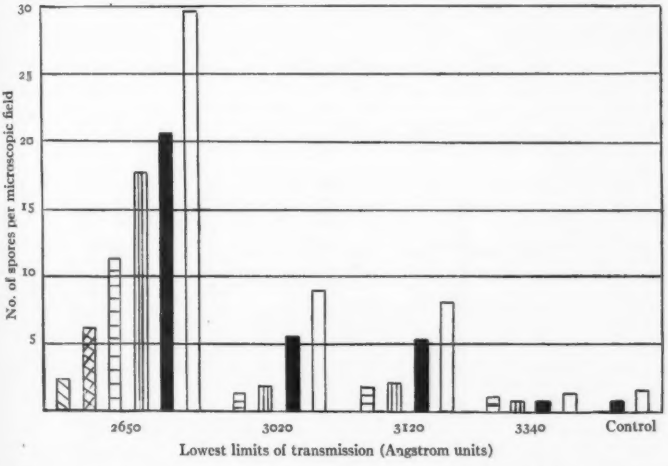

FIG. 11.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through different filters for varying time periods: diagonal lines, 1 day 1 min.; cross lines, 3 days 1 min.; horizontal lines, 1 day 15 min.; perpendicular lines, 3 days 15 min., heavy line, 1 day 6 hours; light line, 3 days 6 hours.

filter transmitting to 3020 A.u., and certainly does not appear photographically in the vitaglass spectrogram (fig. 4 *D*) to be seventy-two times as intense.

It would seem then, that if the difference in intensity between these two filters is primarily responsible for the difference in amount of increase in sporulation, it is the total energy over the whole region of the spectrum transmitted by vitaglass that is responsible and not the energy of any one band.

In the case of long exposures of *Macrosporium*, somewhat better

results were obtained with the glass filters (fig. 12), but even here multiplying the exposure under filter no. 6 by 360 only increased spore production by five and one-third times that of the 1-minute vitaglass culture. An average of eight spores was obtained with an irradiation of fifteen minutes through vitaglass, while an irradiation

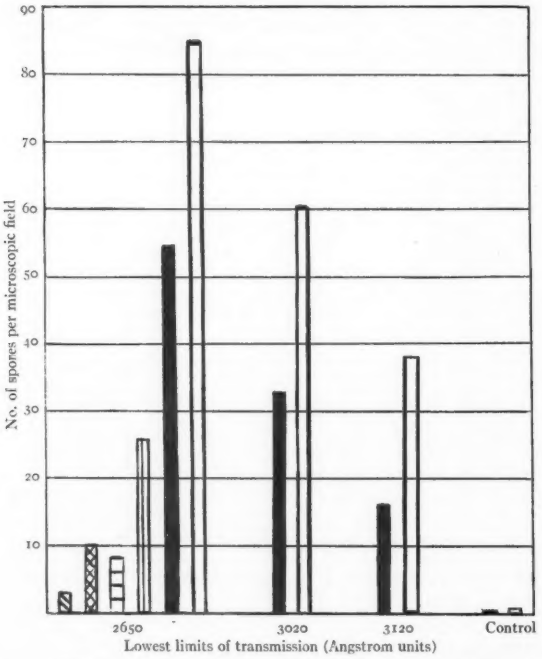

FIG. 12.—Effect on spore production obtained by exposing cultures of *Macrosporium tomato* to quartz mercury arc at 40 cm. through different filters for varying periods of time: diagonal lines, 1 day 1 min.; cross lines, 3 days 1 min. each; horizontal lines, 1 day 15 min.; perpendicular lines, 3 days 15 min. each; heavy line, 1 day 6 hours; light line, 3 days 6 hours each.

twenty-four times as long gave four times as many spores when filter no. 1 was used and twice as many spores with filter no. 6. Another experiment was conducted with *Macrosporium* in which the plates were exposed out of doors to bright sunlight, from about 1:00 to 3:00 P.M. daily for six days (May 3–9), the total exposure being

thirteen hours, fifteen minutes. The shortest detectable lines of the solar spectrum in Chicago at that time of the year are in the region of 3000 A.u., getting as low as 2990 on bright days (BUNDESEN 3). Filter no. 1 (lower limit 3020 A.u.) would therefore be transmitting almost as low as the vitaglass, since the shorter waves transmitted by vitaglass under the arc were not present in the sunlight. Exposures made under vitaglass, thin Jena petri dish (transmitting to 2800 A.u. under the arc), and filter no. 1, all resulted in plentiful sporulation with no detectable difference in quantity. In the control plates held in diffuse light spores were very rare. In a similar experiment with a series of *Fusarium* plates exposed to the sunlight for a total of nine hours on five successive days, more differentiation occurred between exposure under vitaglass and under filter no. 1. At the end of the period of exposure the vitaglass plates showed an exposed ochraceous salmon pionnotes 2.5 cm. in diameter. Macrospores averaged 40 per cent. The plates covered with filter no. 1 showed only a very little pionnotes in the very center, and the macrospores averaged 10 per cent. Control plates in diffuse light showed no pionnotes and only 3 per cent macrospores.

These experiments tend to show that the difference in amount of sporulation under the vitaglass and under glasses transmitting as low as 3020–3120 A.u. is not due solely to a difference in energy of the region from 3020 to 3120 A.u., but the total energy transmitted must be taken into consideration. Intensity is an important factor, for in experiments conducted after the manner of STEVENS (10) it was found that a 4-hour exposure through a spectrograph was insufficient to induce sporulation even under the most intense bands.

Further proof of the effectiveness of ultra-violet radiation in stimulating spore production is found in the following work. During the course of many experiments with *Macrosporium* the controls had been held for several weeks to observe whether sporulation took place as the cultures aged. In all instances it was found that very few spores were formed so long as the cultures were held in the ordinary diffuse light of the laboratory. Likewise cultures that had been irradiated through filters which excluded the rays effective in inducing sporulation have been found to show very few spores after being held for two or three weeks. Many of these plate cultures have been

subjected to irradiation through vitaglass, after they had failed to produce an appreciable number of spores within two weeks. In this manner it has been found that old cultures may be induced to form spores in abundance by irradiation with ultra-violet rays, while companion cultures in diffuse light remained unchanged. Good results have been obtained with 15-minute exposures for three successive days. The spores formed are found throughout the mycelium in the older part of the culture as well as in the younger hyphae along the edges of the plates.

Similar experiments with *Fusarium* were successful in increasing the percentage of macrospores. One exposure of fifteen minutes under vitaglass was sufficient to raise the percentage of macrospores on a plate which had previously been exposed for fifteen minutes on three successive days under filter 586AW (transmitting to 3334 A.u.), from less than 1 to 4.5 per cent. Former control plates exposed at the same time for fifteen minutes under vitaglass increased from 1.2 macrospores to 6.5 per cent macrospores, and plates previously exposed for three days for fifteen minutes to the full arc increased from 2.7 to 8.3 per cent macrospores. In the latter case the vitaglass, while still transmitting the stimulating rays, eliminated most of the lethal rays produced by the direct arc.

Summary and conclusions

1. There is a definite stimulation of spore production in cultures of *Macrosporium tomato* and *Fusarium cepae* on exposure to ultra-violet radiation produced by a quartz mercury arc. Increased spore production appears to be a result of stimulation rather than an indirect result of inhibitory action.

2. Greatest spore production occurs when filters are used whose lower limits of transmission are between 2535 and 2800 Ångstrom units.

3. Slight stimulation results when filters transmitting no lower than 3120 A.u. are used, but those whose lower limits are 3334 A.u. or above are not effective.

4. Stimulation of spore production occurs with radiations of 2535 A.u. and shorter, but with these exposures there is also some lethal effect and inhibition of mycelial development. More killing occurs

with direct exposure to the mercury arc than with exposures through the filters. *Macrosporium* is more resistant than *Fusarium*.

5. Great increase in spore production occurs with radiations of 15–30 minutes. An irradiation of 30 seconds is usually sufficient to induce some increase.

6. Increasing the number of exposures is more effective than increasing the length of exposure. Three 15-minute exposures at 1-day intervals induces more sporulation than one exposure of 45 minutes.

7. Stimulation of spore production is not due to change in the culture medium as a result of irradiation.

8. The increased sporulation is not due to temperature.

9. Control cultures two weeks old, showing no spores, produced hundreds of spores on irradiation.

10. Long exposure to direct sunlight in May through filters transmitting no lower than 3120 A.u. induces abundant sporulation in both *Macrosporium* and *Fusarium*. This shows that if the intensity is great enough, wave lengths shorter than 3120 A.u. are not necessary for stimulation. The experiments with the quartz mercury arc, however, tend to show that the difference in amount of sporulation under vitaglass and under filters transmitting as low as 3020–3120 A.u. is not due solely to a difference in energy of the region from 3120 to 3020 A.u., but to a difference in the total energy transmitted.

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EXPLANATION OF PLATE IV

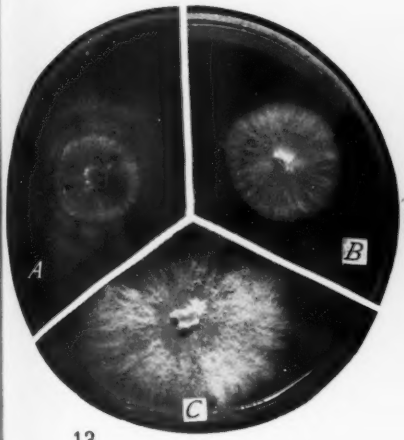
Petri dish cultures of *Macrosporium tomato* and *Fusarium cepae* showing effects of ultra-violet irradiation:

FIG. 13.—*Fusarium cepae: A*, exposed to mercury arc at 40 cm. through vitaglass for 15 minutes; *B*, exposed direct to mercury arc at 40 cm. for 15 minutes; *C*, control grown in diffuse light.

FIG. 14.—*Fusarium cepae*, exposed to mercury arc at 40 cm. for 15 minutes under vitaglass plate, one-half of which was covered with filter no. 6, transmitting to 3120 A.u.: *A*, vitaglass portion (note pionnotes and difference in growth rate); *B*, filter no. 6.

FIG. 15.—*Macrosporium tomato: A*, exposed to mercury arc at 40 cm. for 15 minutes through vitaglass; *B*, exposed direct to mercury arc at 40 cm. for 15 minutes; *C*, control grown in diffuse light.

FIG. 16.—*Macrosporium tomato* exposed to mercury arc at 40 cm. for 15 minutes under vitaglass plate, one-half of which was covered with filter no. 6: *A*, vitaglass portion (note darker color due to sporulation); *B*, filter no. 6 (note grayish color and slightly greater aerial growth).



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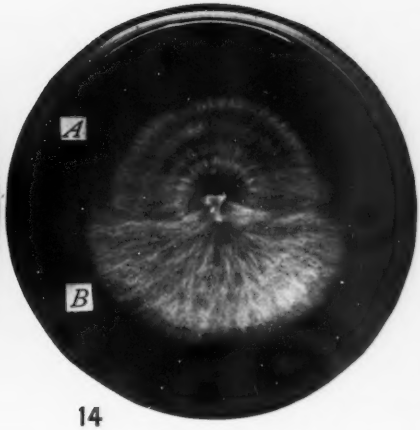

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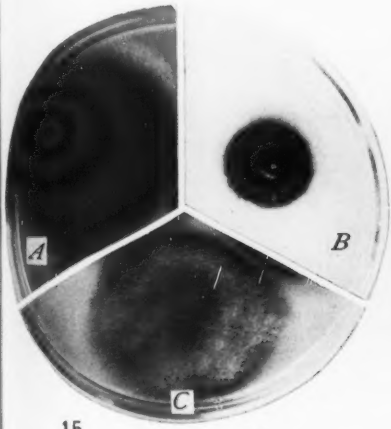

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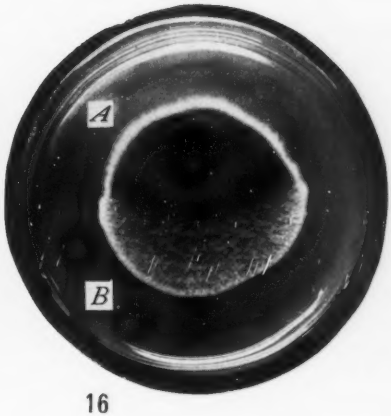


16

RAMSEY and BAILEY on SPORULATION

ORIGIN OF TISSUES OF SCHIZAEA PUSILLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 400

D. R. BARTOO

(WITH TWENTY-SEVEN FIGURES)

Schizaea pusilla (Pursh), native of the pine barrens of New Jersey, is a stranded relic of a past age when tropical plants grew far to the north (MAXON 13). Although at present it is found only in two other regions (Nova Scotia and Newfoundland), there is little doubt that its ancestors flourished in early Mesozoic times. Its local distribution in widely separated regions has long been of interest to the plant geographer, but at present it is agreed that in postglacial times it had a continuous distribution from south to north along the Atlantic coastal plain. The geographic factors accounting for its present limited distribution have been presented by HARSHBERGER (11) and FERNALD (9).

Collections were made October 1, near Forked River, New Jersey, where the plants are found closely associated with those of a typical bog. Surrounded by mats of *Sphagnum*, intertwined with *Lycopodium carolinianum* and liverwort species, the plants are kept constantly moist. Although *Sphagnum* is most prominent, *Drosera*, *Sarracenia*, and *Vaccinium macrocarpon* comprise a considerable part of the association. The most complete description of *S. pusilla* and its habitat is that of BRITTON and TAYLOR (4).

Description

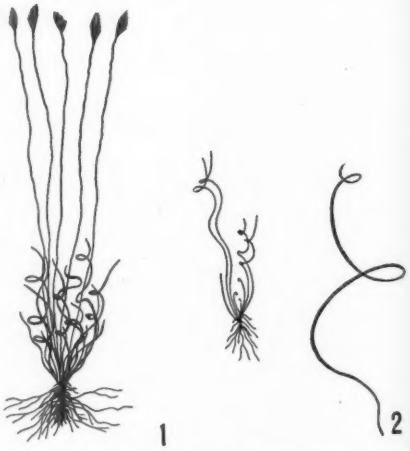
The tiny, upright, perennial rhizome, 5–10 mm. long and 1–2 mm. in diameter, is covered with small, brownish hairs. The roots and leaves arise spirally (acropetally) about the apex from derivatives of segments cut from a tetrahedral apical cell. The rhizome seldom branches (fig. 1).

The linear, tortuous, sterile leaves form a rosette above the rhizome. They are slender and flattened, attaining a length of about 5 cm. The fertile leaves are also slender and flat, and rise to a height of 10–12 cm. above the sterile rosette. They are pinnate at

their extreme ends and each of the five to eight pairs of pinnae bears two rows of sporangia upon its abaxial side, one upon either side of the midrib. Both the sterile and fertile leaves exhibit the characteristic vernation of ferns (figs. 1, 2).

ROOT

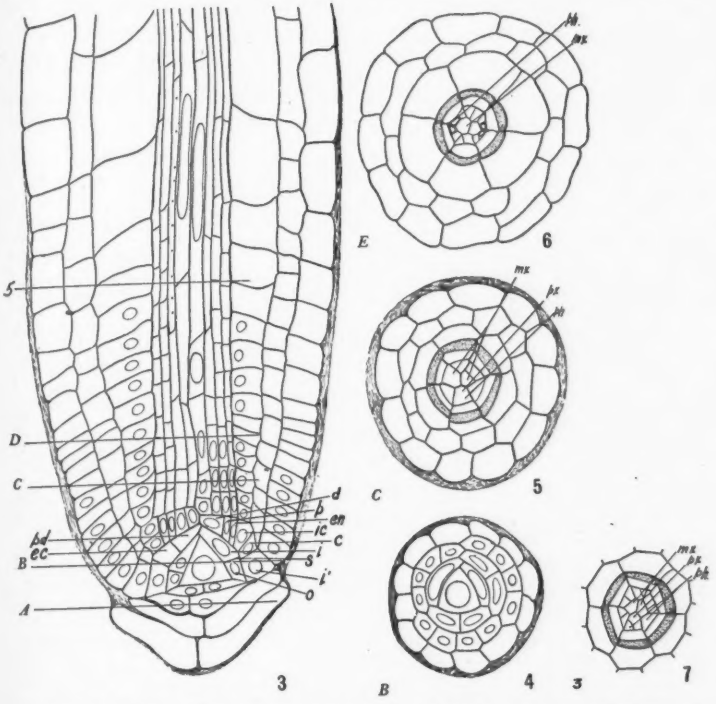
The root has a length of about 5 cm. and a diameter of 0.2 mm., all the tissues of which arise from segments cut from a tetrahedral apical cell (figs. 3, 4). The size of the root and the points of origin of the various tissues correspond to those of *S. rupestris* as observed by the writer (2). The greatest axis of the apical cell lies in the main axis of the root, the three faces directed backward having the form of isosceles triangles. From the derivatives of the segments cut from these three faces the mature tissues of the root are ultimately formed. Segments cut from the forward equilateral face give rise to the root cap (fig. 3). It is obvious that the apical cell and root cap are pushed forward as the segments cut from the three equal faces divide and the resulting cells mature. It is to be noted that in reality segments are not "cut off," but with each nuclear division the protoplasmic mass is divided equally between the segment and the remaining apical cell (fig. 8).



FIGS. 1, 2.—Fig. 1, habit sketch of mature plant, natural size; fig. 2, young plant showing rosette of tortuous sterile leaves, natural size.

Following the cell lineage from one of the three equal segments of the apical cell to the mature tissues of the root can best be accomplished by considering a median longitudinal section through an actively growing root tip (fig. 3). The immediate segment (*s*)

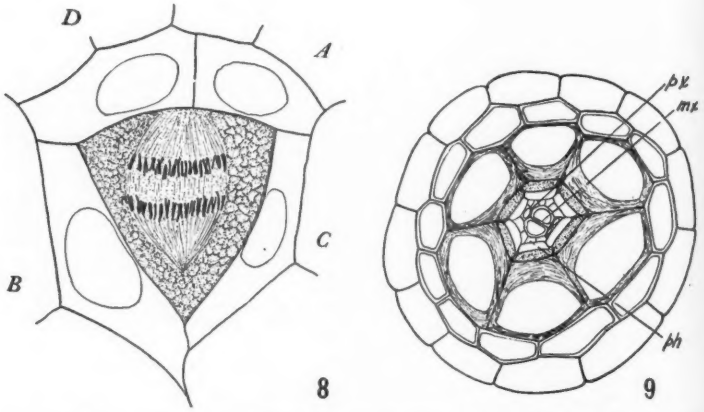
divides by a periclinal wall into an outer (*o*) and an inner cell (*i*). This outer initial cell (*o*) gives rise by one tangential division to the epidermis (*i'*), and to the outer layer of the cortex (*c*). In other



FIGS. 3–7.—Fig. 3, longitudinal median section of root tip: *o*, outer cell of immediate segment; *i*, inner cell; *i'*, initial cell of epidermis; *c*, initial cell of outer layer of cortex; *ic*, initial cell of inner layer of cortex; *en*, initial cell of endodermis; *p*, initial cell of pericycle; *d*, initial cell of desmogen strand; *ec*, mother cell to inner layer of cortex and endodermis; *pd*, mother cell to pericycle and desmogen strand. Fig. 4, transverse section through apical cell region of root tip as indicated by line *B* (fig. 3). Fig. 5, transverse section of root tip in region indicated by line *C* (fig. 3): *mx*, metaxylem; *px*, protoxylem; *ph*, phloem. Fig. 6, transverse section of root tip in region indicated by line *E* (fig. 3). Fig. 7, transverse section of root tip in region indicated by line *D* (fig. 3); stelar region still meristematic.

words, the outer cell of the immediate segment gives rise to the two outer tissue layers of the root. The inner cell, by two successive

periclinal divisions, gives rise to four cells, initiating the inner layer of the cortex (*ic*), the endodermis (*en*), the pericycle (*p*), and the desmogen (*d*). The inner layer of the cortex and the endodermis arise from a common mother cell (*ec*), as do also the pericycle and desmogen (*pd*). The innermost of the four cells derived from *i* varies in its method of division, depending upon its radial position and the tissue to which it is to give rise, as shown in transverse section.



FIGS. 8, 9.—Fig. 8, transverse section of apical cell undergoing cell division; cellular contents equally divided between segment and apical cell; *A*, *B*, *C* indicate order in which last segments were formed; fig. 9, transverse section of mature root: *px*, protoxylem; *mx*, metaxylem; *ph*, phloem.

Simultaneous with the periclinal divisions occurring (fig. 3), walls are formed in radial and tangential planes, as observed in transverse section (fig. 5). Soon after the division of the immediate segment giving rise to an outer and an inner cell, both cells divide radially, giving a sextet of inner cells somewhat tetrahedral in form surrounded by a sextet of isodiametric cells (fig. 26). The division walls of the three segments of *i*, however, passing from near the center of the outer face, do not meet at the common apex of the inner angles. All three walls diverge slightly, either to the right or left, so that the three alternate cells meet at the common center while the remaining alternate three do not extend to the center.

Each of the cells of the outer sextet undergoes two divisions in rapid succession (fig. 27), the first in a periclinal plane giving rise to *c* and *i*, and the second in a radial plane giving twelve cells per tissue layer for the epidermis and outer layer of the cortex (transverse section, fig. 6).

Two of the three alternate cells which meet at the center give rise to metaxylem (*mx*), while the third initiates the protoxylem and phloem (*px*, *ph*, fig. 5). The remaining three cells of the inner sextet (the three which do not occupy positions at the center) give rise to protoxylem and phloem (figs. 6, 7). This arrangement provides a diarch bundle consisting of from two to four protoxylem cells, two metaxylem cells, and from six to twelve phloem cells (fig. 9). Although metaxylem cells do not arise from segments directly opposite each other, they become part of a radially symmetrical bundle (fig. 9). The origins of the tissues of the root correspond to those of *S. rupestris*.

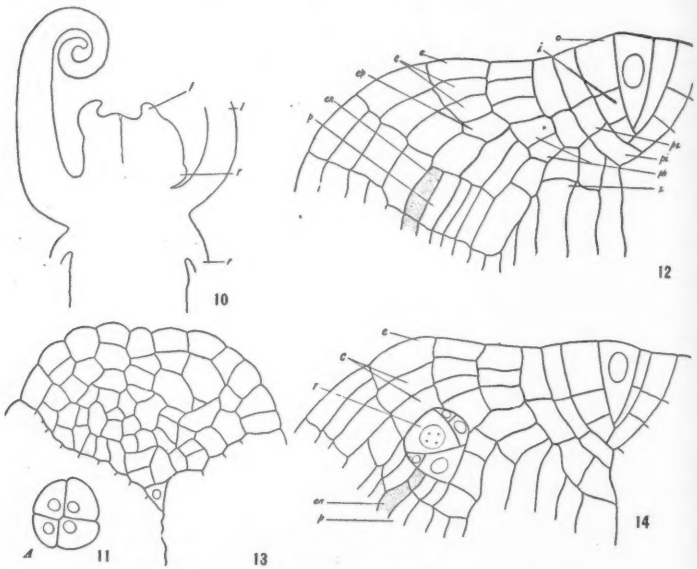
All the tissues of the root are differentiated very early. At a distance of 0.5 mm. from the apical cell all the tissues have been differentiated and cell division in all three planes has ceased. Cell division in a transverse plane ceases very early in xylem and phloem elements. There probably never occurs more than a single division after the xylem and phloem initials have been set apart, and it is very likely that many of these initial cells never undergo divisions in a transverse plane. The mechanics of elongation of the desmogen strand are quite evident. Many transverse divisions in the cortex, endodermis, and pericycle followed by growth of these cells in a longitudinal direction result in a stretching of the cells of the desmogen strand where transverse division has ceased or has never occurred (fig. 3).

Although protoxylem and metaxylem elements can be distinguished by differences in size and position at a distance of 0.2 mm. from the apical cell, it is at a distance of 1.8 mm. that protoxylem thickenings occur. Spiral thickenings occur in the metaxylem at a distance of 2.8 mm. from the apical cell. A definite determination between protophloem and metaphloem could not be made, but thickened walls occur in some of the phloem elements only 0.4 mm. from the apical cell, and it is probable that these represent proto-

phloem elements (fig. 3). No sieve areas were observed in the phloem.

ROOT CAP

Segments cut from the forward face of the apical cell form the root cap (fig. 11). Two successive divisions take place at right angles to each other, so that four cells are formed from each segment.



FIGS. 10–14.—Fig. 10, median longitudinal section through rhizome, showing rudimentary leaves (*l*) and roots (*r*). Fig. 11, transverse section of root cap in region indicated by line *A* (fig. 3). Fig. 12, longitudinal median section through rhizome tip: *o*, outer derivative of immediate apical cell segment; *i*, inner derivative; *e*, epidermis; *c*, cortex; *ep*, mother cell of endodermis (*en*) and pericycle (*p*); *px*, mother cell of phloem and xylem; *pi*, pith. Fig. 13, transverse section through apical cell region of rhizome tip. Fig. 14, same as fig. 12, with root initial (*r*).

Often cells resulting from four or five of the original successive segments remain intact (fig. 3). The successive segments, giving rise to four cells each, usually form their walls in the same planes, but occasionally each alternate segment forms walls which are in planes 45° in a clockwise or counter-clockwise direction as viewed in cross-

section. The large cells from the older segments have scantier contents and are continually sloughed off as the root apex moves forward.

Root hairs

Root hair development begins at a distance of about 3 mm. from the root cap. The epidermal cells giving rise to root hairs (unlike those of *S. rupestris*) elongate in the direction of the axis of the root as the other epidermal cells do. The root hairs persist and were observed on old roots throughout their entire lengths.

Rhizome

The tiny, brown rhizome is upright and attains a length of 6–12 mm. Just as in the root, all the tissues arise from a tetrahedral apical cell, derivatives from its three cutting faces giving rise to the various tissues of the rhizome. Numerous simple hairs arising from the superficial cells of the meristematic region serve as a protective covering for the growing tip. Roots and leaves, which have a spiral arrangement about the rhizome, arise acropetally from its apex.

Two of the three immediate segments cut from the apical cell may be observed in longitudinal section (fig. 12). Each of these divides first into an outer cell and then into an inner. The outer cell by two successive periclinal divisions gives rise to four cells, the outer three of which are initials to the epidermis and cortex. The innermost of these four cells (*ep*), by one periclinal division initiates the endodermis and pericycle. The inner cell of the original segment is divided by a periclinal wall into cells (*px*) and (*pi*), the former of which by one more periclinal division gives the phloem (*ph*) and xylem (*x*) initials. Derivatives of the latter (*pi*) become the pith. Simultaneous with the occurrence of the periclinal walls, anticlinal and radial walls occur, but no attempt was made to follow the sequence of cell lineage as seen in transverse section (fig. 13).

The transitional region of the stelar structure was observed in serial transverse sections. The transition from protostelic to siphonostelic type takes place as described by Boodle (3), and the details will not be repeated here. The number of tracheids of the xylem increases in proportion to the number of leaves and roots. A rhizome having three leaves and two or three roots has sixteen to twen-

ty tracheids near its apex, the arrangement of which is still protostelic. By the time the fifth leaf trace has departed the aspects of a mature siphonostele are evident (fig. 25).

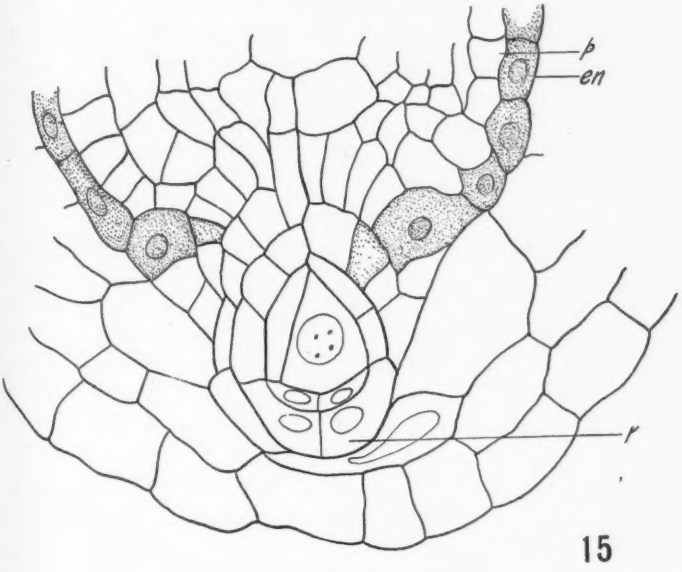
Origin of roots from rhizome

Roots arise acropetally from the apex of the rhizome. They originate from a single cell which is a derivative of the immediate segment of the apical cell. The innermost of the four cells derived from the outer cell may become a root initial (fig. 14). In other words, the cell (*r*) whose sister cells (*ep*, fig. 12) divide to become endodermis and pericycle initiates the root. This root initial when situated only five or six segments from the apical cell of the rhizome becomes considerably larger than its neighboring homologous cells. The first segment is cut off either distal or proximal to the rhizome apex (fig. 14). The formation of this first wall is followed by a second so directed that it meets the first at an angle of 120°. A third wall meeting the first and the second at 120° completes the apical cell of the root. The fourth segment cut from this initial cell forms the root cap. Successive serial segments (either clockwise or counter-clockwise) are cut from the apical cell throughout the life of the root. The derivatives from three sets of immediate segments are differentiated into the various tissues of the mature root, as has already been described. Each tissue layer becomes continuous with the corresponding layer of the rhizome as it differentiates from a common meristematic mass (fig. 14). The apical cell is oriented in such a way that the growth of subsequent derivatives from its immediate segments forces it outward through the cortex of the rhizome. Three or four complete series of segments arise before the root finally breaks through the cortex a short distance from the rhizome apex. As soon as it emerges from the epidermis it turns abruptly downward.

Origin of leaf

The leaves are rather densely spiral as they arise acropetally from the apex of the rhizome. They apparently arise in equal numbers from each of the three sets of outer derivatives of immediate apical cell segments. After the first periclinal division an outer cell (fig. 18), which is set aside as a leaf initial, does not divide peri-

clinally as do the adjacent homologous segments (as seen in transverse section) which are to give rise to tissues of the rhizome. More-



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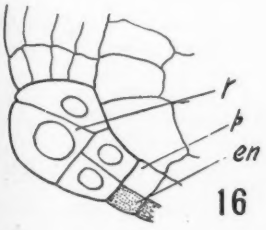

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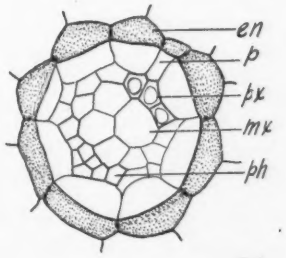

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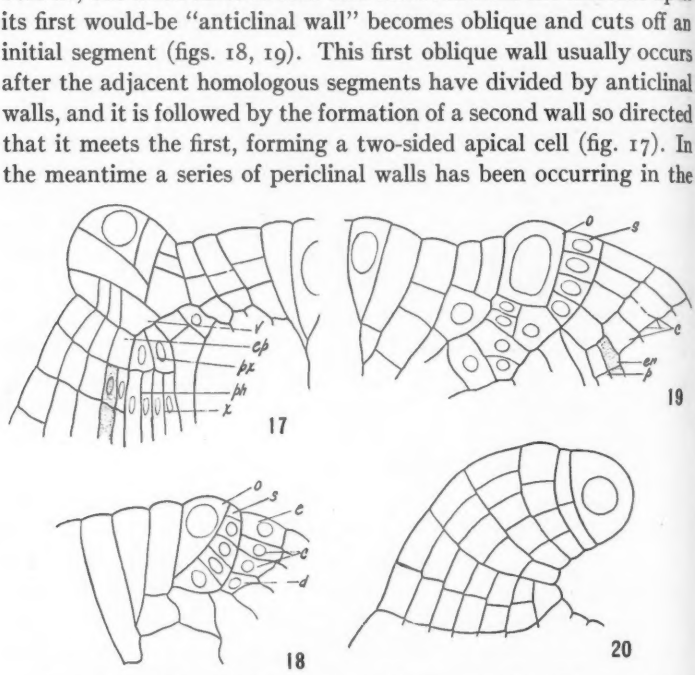
FIGS. 15, 16, 21.—Fig. 15, transverse section through rhizome tip, showing rudimentary adventitious root in longitudinal section: *p*, pericycle; *en*, endodermis; *r*, root cap. Fig. 16, root initial (*r*) in transverse section of rhizome tip; two segments cut from initial root cell; *p*, pericycle; *en*, endodermis; fig. 21, cross-section of rudimentary leaf.

over, this leaf initial seldom divides by anticlinal walls as its neighbors do, but when three or four cells removed from the rhizome apex its first would-be "anticlinal wall" becomes oblique and cuts off an initial segment (figs. 18, 19). This first oblique wall usually occurs after the adjacent homologous segments have divided by anticlinal walls, and it is followed by the formation of a second wall so directed that it meets the first, forming a two-sided apical cell (fig. 17). In the meantime a series of periclinal walls has been occurring in the



FIGS. 17–20.—Fig. 17, rudimentary leaf arising from shoulder of rhizome tip; both leaf and rhizome in longitudinal section: *ep*, mother cell of endodermis and pericycle; *px*, cell showing division which originates phloem (*ph*) and xylem (*x*); *v*, inner cell of immediate segment giving rise to endodermis and stelar structures. Fig. 18, longitudinal section through rhizome tip showing leaf initial (*o*) with single segment (*s*) cut off. Fig. 19, same as fig. 18, showing origin of epidermis, cortex (*c*), endodermis (*en*), and pericycle (*p*). Fig. 20, longitudinal section through rudimentary leaf.

segments of the rhizome which are distal to the leaf initial. Two successive divisions arise in each segment (fig. 12), resulting in four cells the outer three of which become the epidermis and cortex, while the inner cell (*ep*) becomes the initial cell for the endodermis and pericycle. It should be noted that while these segments of the rhizome are undergoing division in a periclinal plane the leaf initial is cutting off oblique segments.

The two successive periclinal divisions of the rhizome seg-

ments are followed by two successive periclinal divisions of the first oblique segments cut off from the leaf initial (fig. 17), and the three outer cells which abut the three outer cells of the rhizome segments form the epidermis and the two cortical layers of the leaf. Thus the epidermis and cortical layers of the leaf and rhizome are continuous. The innermost of the four cell derivatives (*v*) of the immediate segment of the leaf initial corresponds in origin and position to the innermost of the four outer cell derivatives (*ep*, fig. 12) of the immediate segment of the apical cell of the rhizome, and, like it, gives rise to the endodermis and pericycle but in addition gives xylem, phloem, and pith (figs. 12, 17). The endodermis and pericycle, therefore, are probably cortical in origin in the rhizome but of stelar origin in the leaf.

It is obvious that both root and leaf cannot arise from the same immediate rhizome segment, since a leaf initial would include that portion (one-fourth) which becomes a root initial (figs. 14, 17).

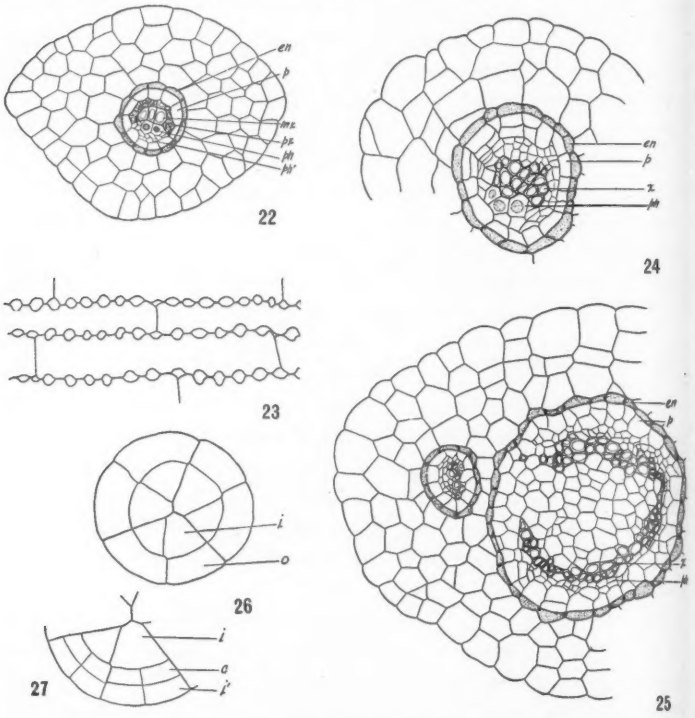
Successive segments cut off from the two-sided apical cell of the rudimentary leaf form a meristematic region which is at first in common with the meristematic region of the rhizome, so that differentiation into various tissues, even though of different origins, takes place simultaneously and the tissue systems of the leaf are from the beginning respectively continuous with those of the rhizome.

Only three or four pairs of segments are cut off from an apical cell of a rudimentary leaf. Each successive oblique wall becomes more nearly perpendicular to the main axis of the leaf, until a would-be oblique wall forms perpendicular to the axis, cutting off a hemispherical cell (fig. 20). It is while in this hemispherical cell stage that the cells of the leaf distal to the rhizome apex begin to grow more actively, causing the leaf to become "circinate" (fig. 20). After twelve or fifteen segments have been cut off from the hemispherical cell it divides into two equal quadrants. Subsequent cell division results in a marginal row of cells which finally lose their meristematic character.

Tissues of leaf

The first tissues of the rudimentary leaf to differentiate are those of the epidermis and two layers of cortex (fig. 17). It has already been noted that the oblique segments cut from the apical cell divide

by two successive periclinal divisions into four cells. The outer three cells undergo no further division in a periclinal plane and become epidermis and cortex. The inner cell (*v*), by a periclinal



FIGS. 22–27.—Fig. 22, cross-section of rudimentary leaf, farther from apical than fig. 21: *ph*, phloem sieve tubes; *ph'*, protophloem. Fig. 23, longitudinal section through mature leaf. Fig. 24, transverse section through rhizome seedling showing protostelic arrangement: *en*, endodermis; *p*, pericycle; *x*, xylem; *ph*, pith cells. Fig. 25, transverse section through mature rhizome showing siphonostelic arrangement; leaf trace has departed from stele. Fig. 26, diagrammatic sketch of region near apical cell in transverse section: *i*, inner cell of immediate segment; *o*, outer cell. Fig. 27, same as fig. 26, showing subsequent divisions of outer cell.

division gives rise to cell *ep*, the common mother cell of the endodermis and pericycle, and to cell *px*, which initiates the phloem, and pith (fig. 17).

The endodermis, which consists of a single layer of ten to twelve cells as seen in cross-section, becomes very conspicuous when only 0.2 mm. removed from the leaf tip, because of its deep staining reactions to safranin. It is quite obvious that these endodermal cells and the adjacent pericycle cells originate from common mother cells (fig. 22).

Just as in the root, the protophloem is the first of the desmogen strand cells to assume thickened walls. First a single cell toward the abaxial side of the leaf becomes conspicuously blue (aniline stain) and somewhat thicker walled at a distance of about 0.25 mm. from the leaf tip (fig. 21). Serial sections show this conspicuous area gradually increasing to the adjacent cells and finally including (at about 1 mm. from the tip) all of the protoxylem cells (fig. 22).

Directly opposite the newly formed protophloem elements (toward the adaxial side) the protoxylem tracheids begin to assume their characteristic thickenings at a distance of 0.7 mm. from the tip (fig. 21). At first there is a single cell; then increasing to three, five, and nine, until at a distance of 1 mm. from the tip from sixteen to twenty protoxylem cells have been formed (fig. 22). It is at about this level that the three metaxylem tracheids become thick-walled. The two large metaphloem sieve tubes soon take on their characteristic blue walls (aniline blue).

It is quite evident, then, that the tissues of the desmogen strand become functional from point of time in the following order: protophloem, protoxylem, metaxylem, metaphloem. At a distance of 1.2 mm. from the tip all the tissues of the leaf are clearly differentiated (fig. 22). The arrangement of the bundle is collateral, as is usual in leaves. The whole consists characteristically of ten to twelve protophloem elements, fifteen to twenty metaxylem elements, three large metaxylem tracheids, and two large metaphloem sieve tubes.

Discussion

The present investigation is largely anatomical. It deals mainly with the origins of tissues and tissue organs. However, much valuable information has been obtained from descriptions and figures of the mature tissues of various species of *Schizaea*. PRANTL (14) describes the mature tissues of the root, rhizome, and leaf of *S.*

penulla, and shows the mode of origin of its sporangium. He "would like to consider" the endodermis as stelar in origin since it and the pericycle arise from common mother cells. He noted that the endodermis and pericycle are sister cells in the smallest strands of leaf veins. PRANTL'S findings are not in agreement with the generally accepted histogenetic idea of HANSON that endodermis is cortical and pericycle stelar in origin. It is also interesting to note that more recent investigations of the origins of tissues in pteridophytes has revealed many exceptions to HANSON'S theory.

BOODLE (3), in his monograph on the anatomy of the Schizaeaceae, deals chiefly with the structures of vascular tissues of the stem and petiole. He noted that there are only three layers of cells outside the endodermis in the root of *S. digitata*, and that the thickenings on the inner and radial walls of the inner cortical layer show distinct stratifications. Because the radial walls of the pericycle, endodermis, and inner layer of the cortex coincide he stated that the pericycle (as well as the endodermis) appears to be cortical.

BRITTON and TAYLOR (4) described the tissue arrangement of the transverse section of a mature root of *S. pusilla*, stating that "there is an endodermis of two layers and that the central cylinder is like that described by PRANTL for *S. penulla*." No doubt the pericyclic layer was mistaken for endodermis; however, in no case have BRITTON and TAYLOR recorded an attempt to trace the tissues to their origins.

TANSLEY and CHICK (15) found the cortex of the stem of *S. malaccana* to be five to eight cells thick. They state that these cortical cells are frequently packed with starch grains and that they always contain considerable mucilage. They noted that the radial walls of the endodermal cells are suberized "in the usual way," and that they are of the same size and correspond to their sister cells of the pericycle. They state that they "can hardly imagine that the sheath layers are not phylogenetically identical in monostelic ferns." In a figure of a longitudinal section through a stem tip (*S. digitata*) they show that the endodermis and pericyle are cortical in origin.

CONARD (8), in his investigation upon *Dicksonia*, found that the endodermis of the root is cortical in origin while the pericycle is

stelar. In the rhizome, however, he showed that both the external and internal endodermis are stelar.

CHANG (7), working upon the origin of tissues in the rhizome of *Pteris aquilina*, found that both pericycle and endodermis are stelar, while NAGELI and LEITGEB (1868) reported that the endodermis of the root of the same species is cortical. The recent work of JOHNSON (12) upon *Equisetum scirpoides*, and of BARCLAY (1) upon *Selaginella wildenowii*, confirms the findings of CHANG in regard to the origin of the endodermis in stems.

GOEBEL (10) states that in the Filicinaceae the leaf primordia proceed from one cell which is a segment of the apical cell. He finds that in those leptosporangiate ferns which have been carefully examined, the leaf primordia have at first a two-sided apical cell. PRANTL (14) shows that the first walls formed in a leaf initial of *Lygodium palmatum* are oblique, forming a two-sided apical cell, which soon becomes hemispherical. CONARD (8) states that the leaf initial in its earliest stages (*Dicksonia*) extends into the stem as far as the future boundary of medulla and plerome, but that no definite order could be discovered in the early divisions of the leaf initial. He reports that in its earliest stages the initial has four cutting faces, which are soon reduced to three and finally to two. In CONARD'S work upon the origin of adventitious roots from the rhizome of *Dicksonia* he found that the root initial arises "in that layer of cells which subsequently gives rise to both endodermis and pericycle." It should be observed that the location of the root initial of *Dicksonia*, as well as the behavior of its earliest cell divisions, forming a tetrahedral apical cell, corresponds to the initial stages of the root of the presently investigated species of *Schizaea*.

BOWER (5) presents a series of figures showing the transition stages from the protostelic to the siphonstelic condition in the rhizome of *Aneimia phyllitidis*. This series corresponds in general to that described by BOODLE (3) for *S. pusilla*, as already noted.

No findings have previously been recorded which show the origins of tissues of roots and rhizomes of the Schizaeaceae. The exact position of the leaf initial and its early subsequent divisions seem to have been undetermined in the Filicineae. The origin of

adventitious roots from a single cell, the sister cell of which gives rise to endodermis and pericycle, and its early subsequent divisions correspond to those of *Dicksonia* as described by CONARD.

Summary

ROOT

1. All the tissues of the root arise from segments cut from a tetrahedral apical cell.

2. The tissues differentiate very early and in the following order: epidermis, cortex, endodermis, pericycle, protophloem, protoxylem, metaxylem, metaphloem.

3. The inner layer of the cortex and the endodermis originate from a common mother cell which is cortical (histogenetic according to HANSON).

4. The pericycle and desmogen strand originate from a common mother cell which is stelar.

5. The root cap formed from segments cut from the fore face of the apical cell remains very simple. Only four cells are formed from each immediate segment.

6. The root hairs persist throughout the life of the root.

7. The root has its origin in a single cell of the meristematic region of the rhizome, the sister cells of which give rise to endodermis and pericycle.

RHIZOME

8. All the tissues of the rhizome arise from segments cut from three faces of a tetrahedral apical cell.

9. The tissues differentiate early and in the same order as do those of the root.

10. The endodermis and pericycle have a common mother cell which is cortical.

LEAF

11. The leaf originates from the first outer derivative of the immediate segment cut from the apical cell of the rhizome.

12. The two-sided apical cell of a rudimentary leaf is transformed into a hemispherical apical cell and finally into a marginal row of meristematic cells.

13. The tissues differentiate in the same order as do those of the root. The endodermis and pericycle have a common mother cell which is cortical.

The writer expresses appreciation for the many valuable suggestions offered by Professor W. J. G. LAND.

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SPIKELETS OF JOHNSON GRASS AND SUDAN GRASS

BETTY LONG

(WITH TWENTY-SIX FIGURES)

Introduction

Johnson grass and Sudan grass are known to be near relatives, but very few of their differences, beyond the fact that the former is a perennial and the latter an annual, have ever been studied. Previous work[1] done on these grasses has been carried on mainly by experiment station workers who were interested in forage values, seeding experiments, chemical analyses, germination, and the eradication of Johnson grass where it had become a pest.

The spikelets of both grasses are arranged in groups, having one sessile and one pedicellate spikelet, or one sessile and two pedicellate in case of a terminal group (figs. 1–3). This grouping of spikelets in twos or threes, or even larger groups, is a more or less constant feature of all the Andropogoneae and Tripsaceae, and it also occurs in other tribes. The structure of these spikelets is very important in a basic study of the Andropogoneae. The flower parts (figs. 4, 5) of both grasses are essentially the same.

The present study has a twofold purpose: to find the morphological differences in the spikelets of the two grasses, and to describe the spikelets of these typical Andropogoneae from serial sections. No such descriptions have ever been published for any of the Andropogoneae, and this study may be used as a basis for future work in this tribe.

History

Johnson grass has been given a number of names (1), among which are *Holcus halepensis* L. (1753), *Andropogon halepensis* (L.) Brotero (1804), and *Sorghum halepense* (L.) Persoon (1805). It has

[1] Since this was written, A. E. LONGLEY has presented a paper at the general section of the Botanical Society of America: Relationship between chromosome number and the annual and perennial habits of grass sorghums. He reports twenty chromosomes for the pollen mother cell of Johnson grass and ten for Sudan grass.

many common names (2), but Johnson grass is the one generally used in the United States.

The native country of Johnson grass is not definitely known. PIPER (9) states that it is "a native of the Mediterranean region

FIGS. 1–5.—Spikelets and flowers of Johnson and Sudan grass: fig. 1, terminal group of Sudan grass having one sessile and two pedicelled spikelets; fig. 2, same for Johnson grass; fig. 3, pair of spikelets of Johnson grass; fig. 4, flower of sessile spikelet of Johnson grass with abaxial lodicules (*lod*); fig. 5, staminate flower of pedicelled spikelet showing aborted pistil (*p*).

from the Madeira Islands to Asia Minor and southeastern Europe." The grass was first described from a specimen collected at the city of Aleppo, from which it received one of its common names and the

root of its specific name. The date of its introduction into this country was about 1830, under the name of *Holcus halepensis*. About 1840 it was cultivated extensively in Alabama.

Andropogon sorghum sudanensis Piper, *A. halepensis sudanensis* Piper, and *Holcus sorghum sudanensis* (Piper) Hitchcock are some of the names given Sudan grass. It is known as "garwari" in Sudan, but upon its introduction into this country was given the distinctive name of Sudan grass (10).

The exact nativity of Sudan grass is unknown, but it occurs in the same regions of the Old World as does Johnson grass, and was long thought to be a form of *Andropogon halepensis*, as Sudan botanists called Johnson grass (8).

The introduction of this grass came as a result of the search to find an annual to replace Johnson grass, which had become a pest because of its rhizomes. In 1909 Mr. R. HEWISON, director of agriculture at Khartum, Sudan, sent seeds, labeled *Andropogon halepensis*, common name "garawa," to PIPER (7).

According to BALL (1), "all cultivated sorghums are held to have been derived from the wild species *Andropogon halepensis*," or *Holcus halepensis* of LINNAEUS. OAKELY (7), in speaking of Tunis and Sudan grasses, states:

> They indicate the possible origin of the cultivated sorghums, resembling on the one hand Johnson grass in organization and vegetative characters, and to a similar degree the cultivated sorghums on the other. That they are primitive forms of sorghums can scarcely be doubted when their characters are carefully studied; and that they are more closely related to the sorghums than to Johnson grass is indicated by the fact that neither possesses underground rootstocks.

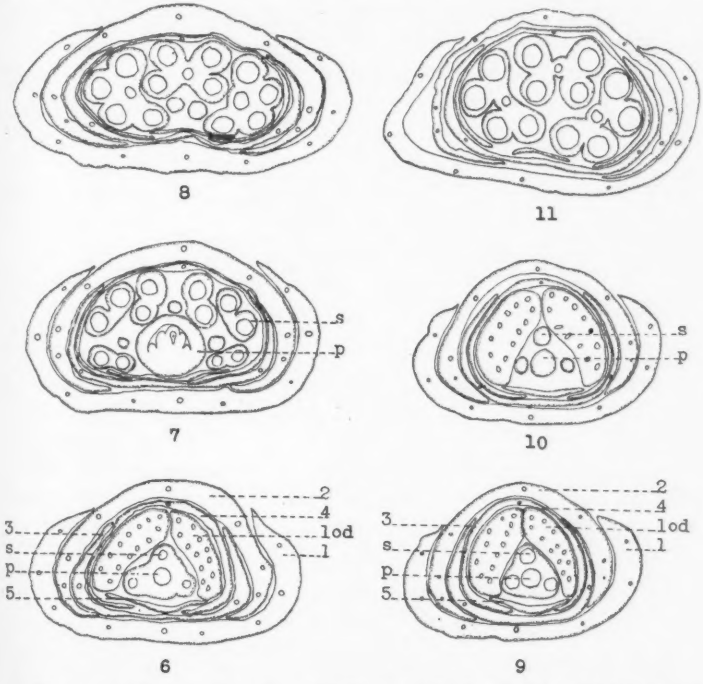
If Johnson grass, Sudan grass, and the cultivated sorghums are near relatives, they might be expected to hybridize. VINALL (10) and OAKLEY (7) say that Sudan grass and the cultivated sorghums hybridize readily. YOUNGBLOOD and CONNER (12) say that Sudan grass and Johnson grass cross. None of these investigators, however, has given any detailed descriptions of the resulting hybrid.

Material and methods

All the material for the study of these two grasses was grown on the University campus during the summers of 1926 and 1927. The Johnson grass was collected from plants grown from a single

rhizome, but the Sudan grass was collected from different plants grown from seeds, which were secured from a seed company.

The spikelets were imbedded in paraffin preparatory to sectioning. Better sections were obtained when the specimens were left only a short time in the higher alcohols, but for a longer period in chloroform and paraffin. Only young spikelets could be sectioned; the more mature ones, after pollination, were too hard.



FIGS. 6–11.—Transverse sections of sessile and pedicelled spikelets of Johnson grass at different levels: figs. 6–8, sessile spikelet; figs. 9–11, pedicelled spikelet; *1*, *2*, *3*, *4*, *5*, bracts numbered from base of spikelet upward; *lod*, lodicule; *p*, pistil; *s*, stamen.

Interpretation of data

JOHNSON GRASS

A cross-section of the sessile spikelet (figs. 6–8) shows that there are five bracts, two lodicules, three stamens, and a pistil. The num-

ber of vascular bundles in the two outer scales is variable and that of the next two bracts is constant; the fifth bract has no bundles in any of the specimens examined. Those of the lodicules are of a different nature from those of the bracts. The first two scales are very clearly the outer and inner glumes. There are four possible interpretations of the third bract, and consequently all the succeeding ones, depending on the number of flowers assumed to be present.

1. If only one flower is present the third bract might be the palea, the fourth the lemma, and the fifth a third lodicule. The two nerves in the third bract and the midrib of the fourth seem to indicate that such an interpretation is correct. This evidence is offset by the fact that bract *3* is on the outside of bract *4* and is attached at a lower level. This also requires the objectionable assumption that the whole floret is turned through an angle of 180°, for the lemma should be on the same side as the lower glume in a one-flowered spikelet. The fifth bract is very clearly not a third lodicule.

2. If only one flower is present the third bract might be the lemma, the fourth the palea, and the fifth a third lodicule. Evidence supporting this idea is that the third bract is on the outside of the fourth bract. Objections to this explanation are that the third bract does not have a midrib, the fourth bract has only one nerve and is not keeled; and the whole flower would still be turned through an angle of 180° as shown by the position of the stamens and lodicules.

3. If two flowers are present the third bract might be the palea of the lower flower, the lemma and flower being absent, the fourth bract the lemma of the second flower which is fertile, and the fifth its palea. Evidences favoring such a condition are the two-nerved third bract; the fourth bract having a midrib and being on the same side as the second glume; the two abaxial lodicules and single stamen on the side of the lemma; and the hyaline nature of the fifth bract. Embryonic remnants of the lost parts should appear below the third bract. The only objection to this interpretation is that the third bract should have its edges turned toward the first bract and not enfold all the higher structures. It is also hard to conceive a palea with no evidence of the lemma subtending the branch on which the palea is borne.

4. If two flowers are present, the third bract might be the lemma

of the first flower, the flower and palea being absent, the fourth the lemma and the fifth the palea of the upper flower. In addition to the evidence supporting this idea that the fourth and fifth bracts are those of a second flower is the fact that the third bract is turned toward the fifth bract and enfolds all the higher structures, which would be expected of a lemma in such a short spikelet. Rudiments of the first flower and palea should appear above the third bract. There is only one inconsistency, the fact that the third bract has no midrib.

The first problem was to find whether the spikelet is morphologically one- or two-flowered. Many spikelets were examined under the binocular microscope but none showed any conspicuous evidence of once having had another flower. On close inspection of the sectioned spikelets under the compound microscope all the specimens are found to have regions of embryonic tissue in a plane above the third bract and below the fifth, or between these in cross-section (fig. 22). This indicates that two flowers were once present, and that the third bract is the lemma of the lower flower.

Although this tissue points to the two-flowered condition, merely finding it does not prove that it is a remnant of the missing flower, but finding a vascular bundle to it and a midrib to the third bract would be more conclusive. In order to learn whether these bundles are present it was necessary to trace all of the vascular bundles in the spikelet.

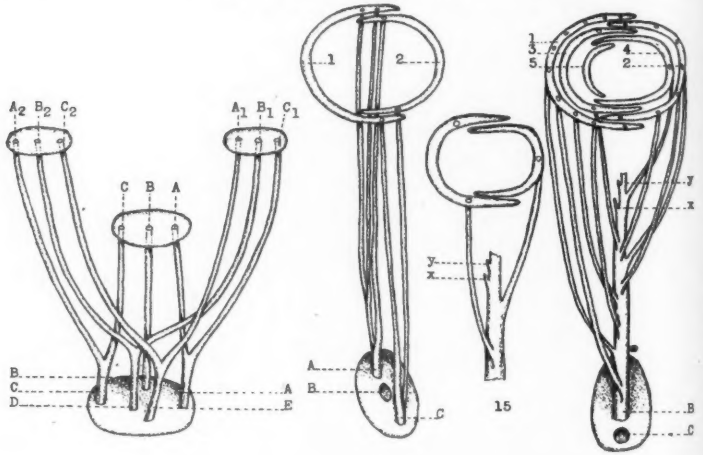
The origin of the bundles, and the levels of branching, have been worked out carefully but will not be described in detail because of their complexity. The main points can be seen from the diagrammatic figures.

Just below the place of attachment of the sessile spikelet on the rachis there are five vascular strands (fig. 23). The strands (fig. 12 A_1, B_1, C_1, A_2, B_2, C_2) of the one or two pedicellate spikelets arise from these. Since those of the pedicellate spikelets branch independently, only those (A, B, C) of the sessile spikelet will be considered.

From fig. 13 it will be seen that strands A and C supply only the extreme lateral edges of glumes *1* and *2*. The bundles from A and C to glume *1* are branched at a lower level than those from B

to the same glume; and the same condition exists with reference to glume *2*. All other bundles of the spikelet have their origin in strand *B* (fig. 14). Two things will be noted: in both glumes the bundles to the right and left of the midrib originate below it; and the bundles to the first glume are attached below those of the second glume.

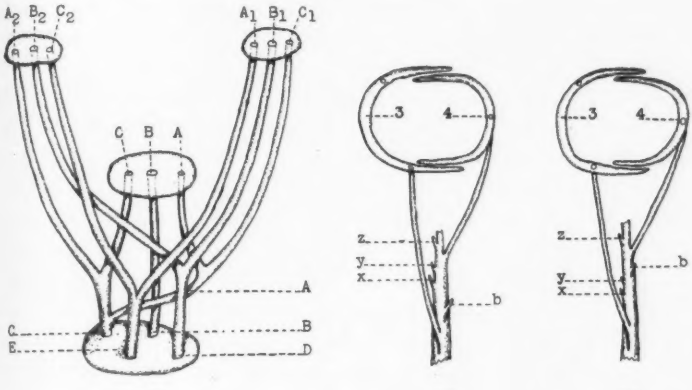
Below the midrib of bract *4* and on the opposite side of the axis is a rudimentary bundle (fig. 14*x*, fig. 24*x*), which is interpreted as



FIGS. 12–15.—Diagrams of vascular system of groups of spikelets of Johnson grass greatly elongated: fig. 12, primary bundles of rachis giving rise to two pedicelled and one sessile spikelets; fig. 13, main bundles supplying lateral edges of glumes only; fig. 14, main bundles supplying all other parts of spikelet; fig. 15, uppermost part of bundle (B_1) supplying pedicelled spikelet (fig. 13 is continuation of bundles *A* and *C* of fig. 12, fig. 14, continuation of bundle *B* of fig. 12); *A*, *B*, *C*, *D*, *E*, primary bundles in rachis; *A*, *B*, *C*, primary bundles in sessile spikelet; A_1, B_1, C_1, A_2, B_2, C_2, primary bundles of pedicelled spikelet corresponding to *A*, *B*, *C* of sessile spikelet; *x*, rudimentary midrib of bract *3*; *y*, rudimentary bundle supplying lower flower once present and subtended by bract *3*.

the midrib of the third bract. Here, as in the glumes, the bundles to the right and left of the midrib are below it and distinctly precede it in order of development; thus *x* is in a normal position in respect to other bundles of bract *3* according to the plan of the glumes, where the lateral bundles are below the midrib. This midrib, to-

gether with the lateral bundles, indicates that the third bract is a lemma, of either a lower or an upper flower.



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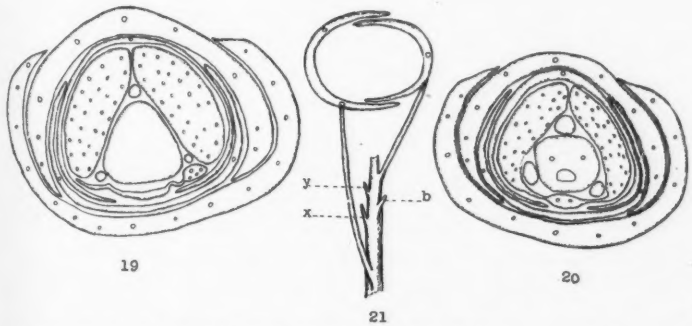
FIGS. 16–18.—Diagrams of vascular system of spikelets of Sudan grass: fig. 16, primary bundles of rachis of Sudan grass corresponding to those of Johnson grass in fig. 12; fig. 17, upper part of bundle *B* in sessile spikelet; fig. 18, upper part of bundle *B* idealized; *b*, rudimentary bundle to left of midrib of bract *4*; *z*, rudimentary bundle to third lodicule once present and still appearing occasionally.



19 20

21

FIGS. 19–21.—Cross-sections of sessile spikelet of Sudan grass and diagram of pedicelled spikelet: fig. 19, cross-section showing third lodicule well developed; fig. 20, same with fifth bract adnate to all three lodicules; fig. 21, upper part of bundle *B*.

A second rudiment (fig. 14*y*, fig. 24*y*) above the midrib of the fourth bract and on the opposite side may be explained as the bundle

to the lower flower when it was present. This indicates that the meristematic tissue represents a lower flower, and consequently that the third bract is a lemma subtending the lower flower.

According to the plan of the glumes, where all bundles to one scale are attached below any of the bundles to a higher bract, x is in a normal position; but contrary to it y of the first flower appears above those of the second floret. In all probability strand B at one time divided into two equal branches, one (now represented by y) to the lower flower, and the other to the upper flower. During evolution, when the lower flower was lost, the rachilla became shortened, and the upper flower moved into a more upright position, so that this rudiment now appears to branch above the bundles of the fourth bract. Or it may be that in reality y branches below this bundle but because the plane of the section is oblique to the rachilla it appears above. The significant point is the order of attachment of bundles in the same bract, however, not the order of those of one bract with respect to those of another bract.

There are two primary bundles to each of the lodicules. In the pistil strand B divides into three parts, two of which enter the style, the third again dividing into four parts and supplying the posterior wall of the pistil and the ovule.

There is sufficient evidence to justify the statement that in the sessile spikelet of Johnson grass the third bract is the lemma of the first (lower) flower, which was lost long ago, and the fourth and fifth bracts the lemma and palea of the second flower which is fertile.

Having established the general plan of the vascular system and the parts of the sessile spikelet, and on the assumption that both spikelets were once perfect, observations were made on the pedicelled spikelet to see wherein these two differ.

The corresponding five bracts are present, offering the same possible interpretations as those of the sessile spikelet; and there are three stamens and two large lodicules (figs. 9–11). In addition, there is an aborted pistil, indicating that the pedicellate spikelet, which is now staminate, was once perfect. In all specimens examined meristematic tissue is present in the same position as in the sessile spikelet, again indicating a two-flowered condition.

In the pedicel there are three large strands (fig. 12 A_1, B_1, C_1)

FIGS. 22–26[2].—Fig. 22, transverse section of sessile spikelet of Johnson grass showing meristematic tissue of first (lower) flower; fig. 23, transverse section below sessile spikelet of Johnson grass showing five primary bundles; fig. 24, transverse section of sessile spikelet of Johnson grass showing rudimentary midrib of third bract (x) and bundle to first flower (y); fig. 25, transverse section of sessile spikelet of Sudan grass showing rudiment of lateral bundle (a) of fourth bract; fig. 26, transverse section of sessile spikelet of Sudan grass showing rudimentary bundle (z) to third lodicule in addition to x and y of third bract and lower flower.

[2]Photographs made by Professor WEATHERWAX. Abbreviations as follows: *mt*, meristematic tissue; *A*, *B*, *C*, *D*, *E*, primary bundles; *x*, rudimentary midrib; *y*, rudimentary bundle of lower flower; *a*, rudimentary lateral bundle; *z*, rudimentary bundle of third lodicule.

which correspond to the strands *A*, *B*, and *C* of the sessile spikelet, except for a few minor differences, so that it will not be necessary to describe the vascular system of the staminate spikelet. Both bundles *x* and *y* appear above the midrib of the fourth bract (fig. 15), but this can be explained by the reorganization of bundles, or the angle at which the section was cut. The fact that *x* is attached in the normal position higher than the lateral bundles to bract *3* is the significant evidence. There is no bundle to the aborted pistil of the upper flower.

From the study of these two spikelets it will be seen that Johnson grass at one time had a group of two perfect two-flowered spikelets, one of which was pedicellate; scales *1* and *2* are glumes, bract *3* is a sterile lemma, and *4* and *5* are the lemma and palea of the functional flower.

Sudan grass

Cross-sections of the spikelets of Sudan grass show all parts corresponding to those of Johnson grass. Because of the similarity of the two species it seems plausible to interpret the third bract as the lemma of a flower that has been lost. Meristematic tissue is present in the same region as in Johnson grass.

In general both grasses have identical vascular systems; so that it will be necessary to mention only the variations which occur in Sudan grass. In the rachis below the sessile spikelet there are usually four vascular strands, one of which divides and the five resulting strands correspond to those of the first species (fig. 16).

At a level slightly above the branching of the lateral nerves of the third bract two rudimentary bundles (fig. 25*a*, fig. 17*b*) are observed and may be explained as the remnants of bundles to the left and right of the midrib of the fourth bract. These rudiments are in their normal position in respect to the midrib of the fourth bract, but not in respect to the bundles of bract *3*.

Immediately above these and on the opposite side of the strand are two other rudimentary traces (fig. 17*x*, *y*). The lower one (*x*) is interpreted as the midrib of the third bract; the other (*y*) is explained as the bundles supplying the lower flower. Both of these bundles are in normal positions in respect to those of bract *3*, but

neither in respect to those of bract *4*. The ideal condition is shown in fig. 18.

The rudiment (fig. 17*z*, fig. 26) above the midrib of the fourth bract and on the opposite side is not present in Johnson grass, but, as will be seen later, it may be the bundle to a third lodicule and is in a normal position.

In Sudan grass there are three primary bundles to each of the two abaxial lodicules, while in Johnson grass there are only two.

Further inspection of twenty-five specimens shows that four have remnants of a third (adaxial) lodicule, which is in all cases adnate to the palea. In three of these the remnant is centrally located in the palea; in the fourth it is more fully developed and is adnate to one side (fig. 19). In all four cases there is a single bundle to this third lodicule. In three specimens this main branch remains as one or divides into two or three smaller branches; in the fourth the bundle divides further and forms many small bundles like those of the abaxial lodicules.

This third lodicule may have a bearing on the phylogenetic significance of the lodicules. Many theories concerning them are current. Miss FRANCIS (3) believes that the flowering scale (lemma) and the palea together constitute a three-parted calyx, the lemma being one part and the palea the other two parts grown together. It would probably follow that the lodicules represent a degenerate corolla. Since we now interpret the lemma as being attached to the rachis and subtending a short branch which bears the palea and the flower, her theory is not widely accepted.

HACKEL, in his earlier work at least (4), regarded the lodicules as equivalents of the palea. In discussing this subject in 1888 (5), he "has endeavored to prove that the anterior scales represent halves of a leaf which sometimes remains undivided, and can be regarded as a second, and the posterior scale as a third, palea." By anterior scales he means the two abaxial lodicules; the third which is sometimes present is adaxial. He explains that the difference in structure is due to physiological functions.

HITCHCOCK and many others apparently consider the palea as homologous with the prophyllum of vegetative parts of the plant. "The lodicules are interpreted by some to be homologous with the

divisions of a perianth of which only two divisions have usually persisted" (**6**).

If the third lodicule were explained according to HACKEL, it would be seen that the two abaxial lodicules forming the "second palea" in this case would have six primary vascular bundles; the "third palea," which is the third lodicule, would have one vascular bundle which should be attached above those of the second palea. In all cases the palea is adnate to both abaxial lodicules in low levels (fig. 20) and also to the third lodicule. It would be a very peculiar condition in which the first palea was attached at both sides to the divided second palea which was in a higher plane, and also to the third palea which was in a second higher plane. It is no more correct to interpret the abaxial lodicules as a palea because they are united in some grasses than it would be to say that in the case of Sudan grass the adaxial and one of the abaxial lodicules form a palea because they are united.

The other explanation seems more plausible. The palea may be adnate to the abaxial lodicules in low levels because of the crowded condition of the spikelet. It is not inconsistent that the adaxial lodicule, which is in the same plane with the abaxial ones, is attached to them and to the palea also. In those cases where the third lodicule is centrally located in the palea, these two structures seem never to have been differentiated at the growing point.

Cross-sections of the pedicellate spikelets show all the parts corresponding to those of the pedicelled spikelet of Johnson grass. All specimens have embryonic tissue above the third and below the fifth bracts. In contrast with the sessile spikelet of Sudan grass there is no evidence of a rudimentary lodicule.

The vascularization of this spikelet is essentially the same as that of the sessile spikelet below the third bract; above this there are a few differences (fig. 21). The rudiments x and y are in their normal positions in respect to bract 3; but those of the first floret are not normal in relation to those of the second floret.

Summary

1. In both spikelets of both grasses the vascularization is essentially the same. The nerves to the right and left of the midrib in

every bract are attached below the midrib of that bract. All the bundles to one bract are attached below and on the opposite side of the rachis from any of those to the next higher bract.

2. Meristematic tissue of a first flower and a rudimentary bundle to it establish the fundamental two-flowered condition in both spikelets of both grasses. This is consistent with the now generally accepted opinion as to the nature of this spikelet. It is also consistent with the structure of the spikelet in the Paniceae, where both the lemma and palea of the lower floret are present, and a staminate or even a perfect lower flower is sometimes present; and in the Tripsaceae, where the same condition has been established.

3. The rudimentary midrib to the third bract and embryonic tissue above it establish the fact that the third bract is a lemma of a lower flower.

4. The aborted pistil suggests that the pedicelled spikelets of both grasses once had a perfect flower.

5. Some of the spikelets of Sudan grass have a partly developed adaxial lodicule; in the others this lodicule is represented by a rudimentary bundle.

6. Observations on the lodicules of Sudan grass seem to indicate that they are members of a perianth, all three of which are present in a few cases.

I wish to express appreciation for the CLARA JAVAN-GOODBODY Scholarship (1927–28) and a special fellowship (1928–29) which made this study possible; and for the valuable suggestions and encouragement given by Professor PAUL WEATHERWAX, under whose direction the work was done.

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EFFECT OF LOW TEMPERATURES ON GERMINATION OF IMPERMEABLE SEEDS[1]

W. F. Busse

Introduction

The problem of hastening the germination of seeds having a dormancy due to an impermeable seedcoat has received considerable attention, since it is of both scientific and economic importance (2, 5). Among the various treatments that have been suggested, removing the seedcoat is one of the most direct and obvious methods that can be used. This may be done mechanically or by means of concentrated sulphuric acid, but both methods have very apparent disadvantages. Scratching or scarifying the seeds will cause many of them to become permeable, but with large irregular seeds like alfalfa this treatment injures many, and it also decreases their keeping power (4).

Recently Davies (3) found that subjecting clover and alfalfa seeds to pressures of 500–2000 atmospheres reduced the proportion of impermeable seeds. Staker (10) and Stewart (12) found that dry heat reduced the impermeability of alfalfa seeds. The alternate freezing and thawing which seeds receive when they are planted in the late fall causes some to become permeable (8), but the freezing also kills the young plants and seeds which have imbibed much water. Midgley (7) tested the effect of alternate freezing and thawing on hard seeds. He found the first freezing of moist alfalfa seeds to about $-5°$ C. caused some of them to become permeable, but subsequent freezings had little effect in reducing the impermeability. If the seeds were kept moist for several months, he found that as many seeds would become permeable without freezing as with freezing, and he makes the statement that the intensity of freezing had no effect on the increase in permeability.

In the course of some investigations of hard seeds carried out in

[1] Contribution from the Laboratory of Physical Chemistry of the University of Wisconsin.

this laboratory, the writer was led to study the effects of very intense freezing on seeds, down to the temperature of liquid air (−190° C.), with results which were rather surprising. It has long been known that dry seeds stand freezing in liquid air (1) and seeds having a high moisture content are killed by this treatment (11), but very little work has been reported on the changes other than killing which are produced when the seeds are frozen at very low temperatures. It was found in this work that while freezing the dry impermeable seeds at moderately low temperatures had no effect on their germinability, freezing them at very low temperatures would enable the seeds to germinate promptly when placed under normal germinating conditions. In the case of some sweet clover seeds, freezing in solid carbon dioxide snow (−80° C.) had almost no effect on the impermeable seeds, but freezing in liquid air was very effective in destroying the impermeability without injuring the seeds. With alfalfa seeds, freezing to −20° C. had little effect, but freezing to −80° C. made nearly all of them permeable. These results were deemed of sufficient importance to justify a careful study of some of the factors involved, so the following experiments were performed.

Experimentation

Several different lots of air-dry alfalfa, common sweet clover, and Grundy County sweet clover seeds, each of which contained a high percentage of impermeable seeds, were used in these experiments. The seeds were of the 1927 crop, and most of the tests were made during the winter of 1927–28, with the aging tests running through the following year. The alfalfa was machine hulled, but the clover seeds were hulled and cleaned by hand.

To treat the seeds, they were usually put in a small test-tube and this was then immersed in the refrigerant, which was contained in a Dewar flask. The seeds were then either given a rapid, non-uniform warming by pouring them from the test-tube onto a warm metal plate (treatment I), or else they were allowed to warm up very slowly by leaving them inside the Dewar flask until the refrigerant had evaporated and the flask had warmed to room temperature (treatment II). In a few cases the seeds were cooled comparatively slowly by suspending them several centimeters above the surface of

the refrigerant in the Dewar flask for several hours, while in other cases the seeds were poured directly into the refrigerant. In one experiment seeds were left in liquid air continuously for about six months. The germination tests were usually made by putting 100 seed samples on filter papers in crystalizing dishes, and keeping them moist with distilled water. The treatments and tests were repeated several times to check the results. A few samples of treated seeds were grown in soil in the greenhouse and it was found that the plants were perfectly normal.

1. Freezing in liquid air

1. Effect on different seeds.—Table I, showing the effect of freezing several lots of seeds in liquid air, is typical of the results obtained with this refrigerant. The seeds given treatment I were

TABLE I

Effect of freezing in liquid air on germination

Seed	Percentage germination		
	Control	Treatment I	Treatment II
Grundy County sweet clover, Lot 1	14	76	90
Grundy County sweet clover, Lot 2	47	70	70
Common sweet clover, Lot 1	64	86	94
Common sweet clover, Lot 2	58	83	.....
Alfalfa, Dakota	68	100	.....
Alfalfa, Idaho	52	96	100

kept in the liquid air from three to five minutes and then rapidly warmed, while the seeds given treatment II were slowly warmed.

The increase in germination from 14 to about 90 per cent found with Grundy County sweet clover shows the effectiveness of this treatment. The somewhat lower germination of the seeds that received treatment I is probably due to the fact that they were not kept in the liquid air long enough for all the seeds to be completely cooled. If the seeds that did not grow were given another freezing in liquid air, from 20 to 100 per cent of them would become germinable.

The length of time the seeds are kept at the liquid air temperature has almost no effect on the germination, at least for times of

cooling up to six months. This was shown by putting some sweet clover seeds into a flask that was kept filled with liquid air, and withdrawing seeds for germination tests every few months. Table II shows the results obtained.

The seeds kept in liquid air for 176 days were not tested until nearly six months after they were removed. Since the control gave a germination of only about 50 per cent at that time, it indicates that storing in liquid air is not injurious, and it even may be beneficial in retarding normal aging of the seeds. If life is a dynamic

TABLE II

EFFECT OF TIME OF COOLING IN LIQUID AIR ON GERMINATION OF SWEET CLOVER SEEDS

TIME IMMERSED IN LIQUID AIR	NUMBER TESTED	PERCENTAGE GERMINATION
0	100	64
2 minutes	100	80
35 days	50	76
35 days	50	84
90 days	50	72
176 days	50	74

process which obeys the laws of chemical reactions, then these reactions take place at an enormously reduced rate at this temperature. If the usual temperature coefficient of between two and three for a 10° temperature change holds over this range, a simple calculation will show that their reactions are reduced to between one-millionth and one-billionth of their velocity at normal temperatures. For seeds at this temperature, a thousand years are but a day so far as the life processes are concerned.

2. EFFECT OF REPEATED COOLING IN LIQUID AIR.—It was thought that if the rapid cooling to these low temperatures was causing any injury to the seeds, this would be increased by repeated cooling and warming. To test this, samples of Grundy County sweet clover, giving a germination of about 24 per cent for the untreated seeds, were repeatedly cooled and warmed as rapidly as possible by putting them into small (2×0.5 inches) test-tubes. These were immersed directly in the liquid air and then the seeds were warmed by pouring them out in a thin layer on an aluminum plate. After a

single treatment the germination was raised to 85–90 per cent; after five such treatments the germination was 92 per cent; and after twenty treatments the germination was 85 per cent (50-seed samples).

To make an even more drastic test, some alfalfa seeds in a small test-tube were covered with water and the test-tube then immersed in liquid air. After the seeds and ice had reached the liquid air temperature, the test-tube was removed and the ice allowed to melt. The seeds were then removed from the water and dried. When put on the moist filters they showed 100 per cent germination, but during germination the seed coats cracked or broke in several places, showing that they had been weakened by the treatment.

TABLE III

EFFECT OF FREEZING IN LIQUID AIR ON CHANGE OF GERMINATION ON STORING

SEEDS	PERCENTAGE GERMINATION			
	Initial	Six months	Nine months	Twelve months
Alfalfa, control	50	.....	72	.....
Alfalfa, treated	95	.....	92	.....
Sweet clover, control	47	41	.....	40
Sweet clover, treated	70	70	.....	74

3. CHANGE OF GERMINATION ON STORING AFTER FREEZING IN LIQUID AIR.—Since many of the treatments now in use to increase germination of impermeable seeds also injure their keeping power, storage tests were run on treated and untreated seeds to see whether freezing also injured the keeping power. The tests have been run for a year without showing any great deterioration of the seeds. The results of a typical experiment with alfalfa and sweet clover seeds which had been frozen in liquid air are shown in table III.

The low germination of the sweet clover is due to the presence of many immature seeds in this particular lot. The seeds were kept in the laboratory in paper envelopes or in flasks closed with cotton so that the air could reach them, to duplicate the usual storage condition as closely as possible. The results showed that freezing in liquid air has no appreciable effect on the keeping power of the seeds.

In some germination tests, both on filter papers and in soil, it was found that the freezing not only increased the germination, but it also stimulated the growth of the seedlings. This may be due simply to the fact that water reaches the embryo more readily, but it is also possible that the liberation and the distribution of the growth enzymes are accelerated by the freezing.

II. Effect of freezing seeds in CO_2 snow

Some of the beneficial effects of liquid air on germination of seeds can be produced by the less intense freezing obtained with solid carbon dioxide. The results of the test on dry seeds are given in table IV, where treatment I consisted in cooling with the CO_2 snow for 10 minutes, and then rapidly warming the seeds; while treatment

TABLE IV

Effect of freezing in CO_2 on germination

Seeds	Percentage germination		
	Control	Treatment I	Treatment II
Alfalfa, Idaho	62	86	87
Alfalfa, Dakota	61	70	.....
Sweet clover, Grundy Co., Lot 1	14	22	.....
Sweet clover, Grundy Co., Lot 2	53	55	54
Sweet clover, common, Lot 1	63	72	76
Sweet clover, common, Lot 2	58	68	.....

II consisted in cooling the seeds, and then letting them slowly warm within a Dewar flask.

It is seen that there is comparatively little change in the germination of clover seeds with this treatment, but the germination of alfalfa seeds is very distinctly improved. The seeds may be given a superficial coating of moisture before freezing, or they may be frozen repeatedly with beneficial effects on the germinability and no bad effects on the keeping power, as is shown by table V.

Freezing these seeds in liquid air raised the germination to 98 per cent. These results show that even repeated freezing in CO_2 snow under drastic conditions does not appreciably affect the keeping power of the seeds. Another lot of alfalfa gave an initial test of 60 and 85 per cent for the control and the treated samples respectively, and twelve months later the germination test of the same samples

was 92 and 94 per cent respectively. In this case the untreated seeds lost their impermeability on standing, but even here the treatment had no injurious effect on the seeds.

III. Freezing to −20° C.

Some lots of alfalfa had their germination raised about 12 per cent by treatment, but this was only one-third of the effect produced by freezing the same seeds in solid CO_2. Clover seeds were not affected by freezing to −20° C.

TABLE V

Effect of freezing alfalfa seeds in solid CO_2 under various conditions

Treatment	Percentage germination		
	Initial	Two months	Ten months
1. Control	53	60	72
2. Frozen once	86	96	90
3. Frozen five times	98	100	.....
4. Seeds wet, then frozen once	92	90	88
5. Seeds wet, frozen four times	99	92	96

IV. Specific heat of alfalfa seeds

Because of the possible practical application of these results, experiments were carried out to determine the specific heat of the seeds down to −190° C. This was done in two ways. In the first method a 500 cc. Dewar flask, half filled with fresh liquid air, was placed on a large analytical balance, and the weight of the flask and air was recorded every minute for 15 minutes. Then a weighed amount of alfalfa seeds was poured into the flask and when they had cooled off, the weight was again recorded every minute. The weight was then plotted against the time, and by extending the first and last curves to the time the seeds were added, the weight of liquid air evaporated by the seeds could be determined. The average of several experiments showed that 5 gm. of alfalfa seeds would evaporate 4.3 gm. of liquid air. Since the latent heat of vaporization of liquid air is 50 cal./gm. (**9**), 1 gm. of seeds give up $4.3 \times 50 \div 5$, or 43 cal. in cooling from 20° to −190° C., so their average specific heat over this range is $43 \div 210$, or 0.21 cal./degree.

This value was checked by measuring the volume of air evapo-

rated by a known weight of seeds. A Dewar flask containing liquid air was tightly closed by a 3-holed stopper. One tube through this stopper went to a large gas burette, a second was connected by a rubber hose to a test-tube containing a weighed quantity of seeds at room temperature, and the third tube had a stopcock opening to the atmosphere. To make a determination of the specific heat, the stopcock was closed so that the air which vaporized in the Dewar flask had to go into the gas burette, and the volume of air in the burette was recorded every few minutes to get the normal rate of vaporization. After this was obtained the test-tube containing the seeds was inverted so that the seeds fell into the liquid air, and the test-tube was then returned to its original position. The transfer of the seeds thus took place without opening the system or changing its volume. The volume was again read every few minutes to get the final rate of vaporization. By extrapolating the two volume-time curves to the time at which the seeds were added to the liquid air, the volume of air evaporated by the seeds was obtained. The average of four determinations gave 700 cc. of air at room temperature and pressure evaporated by 1 gm. of seeds. Taking the weight of air under these conditions as $1.2 \cdot 10^{-3}$ gm. per cc., 1 gm. of alfalfa seeds vaporized 0.84 gm. of liquid air, which gives the seeds a specific heat of 0.20 cal./degree. This checks the other value rather well.

In these experiments no attempt was made to utilize the cooling effect of the vapors coming from the liquid air. If one takes the molal specific heat of air between $-190°$ and $20°$ C. as 6.5 cal. per degree, then the heat absorbed when 1 gm. of air warms from $-190°$ to $20°$ C. is $6.5 \times 210 \div 30 = 47$ cal., which is more than the heat absorbed in vaporizing 1 gm. of liquid air.

If this could be used in cooling the seeds it would reduce the amount of liquid air required by about one-half, but even under these conditions it would hardly be profitable to use liquid air to increase the germination of clover seeds commercially, since each pound of seeds would require from one-half to one pound of liquid air. However, when dealing with special experimental lots of seeds, where the ordinary procedure would be to scratch or prick each seed by hand, this method of freezing may be found very useful and convenient. Because of the high latent heat of vaporization and the

low cost of solid CO_2, it might be not only convenient but also very profitable to use this refrigerant to freeze lots of alfalfa which contain a high percentage of hard seeds.

Discussion

The increased germination obtained after freezing clover and alfalfa seeds at very low temperatures is probably due to the formation of very tiny cracks in the impermeable membrane. This process seems to depend on at least two factors, making the membrane brittle by the cold and at the same time exerting forces tending to burst or warp it, due to the different coefficients of expansion of different parts of the seed. The extent of these forces will be determined by the size, shape, structure, and composition of the seed, the amount of water in it, and the rate, uniformity, and amount of the cooling. Some idea of the forces involved may be obtained from the fact that when castor beans or flax seeds are immersed in liquid air and then warmed rapidly and non-uniformly by placing them on a warm metal plate, they often explode with a force sufficient to rip off the outer seed coats and send the seeds flying a distance of 5–10 feet. Any treatment which magnifies these forces, such as increasing the temperature drop, either through the use of a colder refrigerant or starting with the seeds at somewhat above room temperature, tends to increase the effectiveness of the treatment. Other things, such as repeated freezing, or giving the seeds a superficial coating of moisture before freezing also helps, but their effects seem to be less than that due to cooling the dry seeds to $-180°$ C.

The fact that even repeated freezing to $-80°$ C. and rapid thawing of seeds having a superficial coating of moisture do not injure their keeping power shows that the changes produced are very minute. It seems probable that the permeability of the seed coat for oxygen is not changed much by this treatment, but it would be interesting to see whether intensive freezing destroyed the dormancy of cocklebur seeds, which is known to be due to the presence of a membrane impermeable to gases. It is possible that seeds which normally require alternating temperatures for germination might grow if kept at a constant temperature after they had been given a sufficient intense freezing. One would not expect seeds having a

dormancy due to an embryo condition to be made germinable by this treatment, and it was found that dormant belladonna and *Hyoscymus* seeds were not made germinable by freezing in liquid air.

Since the dormancy of most alfalfa seeds can be broken by freezing around −80° C., and temperatures in this range can be produced very cheaply by the use of solid CO_2, liquid ammonia, etc., it may be worth while to give this treatment to all lots of alfalfa which contain many impermeable seeds.

Summary

1. Dormancy of seeds due to an impermeable seed coat may often be broken by cooling the seeds to very low temperatures.

2. Impermeable sweet clover and alfalfa seeds may be made permeable without injuring, by freezing the air-dry seeds in liquid air (−190° C.).

3. Sweet clover seeds were not injured by keeping them in liquid air for nearly six months (175 days); and rapidly cooling seeds down to −190° C. twenty times, followed each time by a very rapid warming, did not injure them.

4. Cooling to −80° C. caused most of the impermeable alfalfa seeds to become permeable, but this treatment had very little effect on impermeable sweet clover seeds.

5. Freezing air-dry seeds to very low temperatures had no appreciable effect on their keeping power.

6. The average specific heat of air-dry alfalfa seeds between 20° and −190° C. is about 0.21 cal. per degree.

7. The process of destroying the impermeability of alfalfa seeds by intense freezing appears to be a practical one for large-scale operations.

The writer wishes to express his appreciation to Professor F. DANIELS for the constant inspiration he has furnished throughout the work, and to the members of the botany and agronomy departments for the help they have given.

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SPERMATOGENESIS IN COLEOCHAETE SCUTATA

OPHELIA C. WESLEY

(WITH PLATES V, VI)

Introduction

This paper is a report of the continuation of the work outlined in a former paper (5). PRINGSHEIM (4), OLTMANNS (2), and JOST (1) have studied the spermatogenesis of one or more species of *Coleochaete*. As early as 1860, PRINGSHEIM described the spermatogenesis of *C. scutata*, so far as the process could be observed in whole mounts. So far as I have ascertained, no sections of the thalli bearing antheridia have ever been studied. The purpose of the present paper is to report the results of a study of such sections, together with a few observations made upon surface views.

Material and methods

The major part of the material for this work was collected at Miller, Indiana. The collections were made at one- and two-week intervals from June 18 until October 30, 1927. Some information, however, was obtained from material collected at weekly intervals from a lagoon in Washington Park, Chicago, beginning July 6 and continuing until November 30, 1926, after which monthly collections were made until April, 1927. The best developed thalli were found on *Typha* leaf sheaths of the previous year's growth. The material collected in Washington Park was on *Sagittaria*, *Typha*, *Nymphaea*, and *Castalia;* none was found on sticks, stones, or other aquatic plants than those mentioned. In collecting about Madison, Wisconsin, however, *C. scutata* has been found also on the leaf sheaths of *Sparganium* and on the leaves of *Potamogeton*.

The material differed in no way from that described in the previous paper (5), except that a few sections were stained with Flemming's triple stain. The greater part of the material was stained in Haidenhain's iron-alum haematoxylin. Sections were cut 3–7 μ in thickness.

Spermatogenesis

TIME OF FORMATION.—When the first collection of material was made, June 18, 1927, a number of thalli showed that the first division of a vegetative cell in preparation for the formation of antheridia was complete. At this time female plants were found in which the zygotes were completely covered with dark brown cells. These zygotes were almost if not quite mature; hence antherozoids must have been produced much earlier than the collection date. Eggs in some of the thalli had disintegrated, probably because of lack of fertilization. In all the material collected at Miller, the number of abortive eggs was high in comparison with the number of mature zygotes. Very few thalli produced zygotes, and those having them had very few.

In the material collected in Washington Park, antheridia and oogonia did not begin to appear until about the middle of September. Since the two places are only a few miles apart, it seems surprising that antheridia and oogonia should be produced in one location so much earlier than the other.

The thalli producing antheridia on June 18, 1927, were all found on *Typha* leaf sheaths of the previous year's growth. As soon as the ice melts in the spring, zoospores begin to form in the thalli that have over-wintered. This time of formation of zoospores comes long before the new vegetation begins to appear. *Typha* is more resistant to the forces bringing about its decay than is *Sagittaria*, hence the old *Typha* stems wrapped in their leaf sheaths are rather well preserved at this time. Zoospores come to rest upon these leaf sheaths, and thalli begin to develop. This early development gives them the start over those that are later formed on the fresh living plants. Since these early thalli reach maturity before the others, it is natural for them to begin to form antheridia and oogonia before those developing later.

According to WEST and FRITSCH (6), *Coleochaete* produces antheridia and oogonia in England from May to June. OLTMANNS reports sexual reproduction for the "Titisee" region from September to October. He says (3) that the time of sexual reproduction depends upon the locality, implying that differences in temperature are the effective factors. In the region about Chicago, however, no such

range of temperature could account for the two months' difference in time in the production of antheridia. Miller is only thirty miles from Chicago; hence there could be no such difference of temperature as one finds between England and Germany.

Antherozoids ready to be shed, as well as cell walls from which they had escaped, were found in the material collected June 27, 1927. The number of antheridia increased until the middle of September, then gradually declined until the latter part of October. A few were found as late as October 30 (1927).

No swimming antherozoids were observed; hence the time of their escape was not determined. The time of day at which the division by which antheridia are formed takes place was likewise not determined. Material was killed at intervals from 7:00 A.M. to 6:00 P.M. (fig. 1), however, and only prophases were found. From this fact one would infer that the division took place between 6:00 P.M. and 7:00 A.M.

PLACE OF FORMATION.—Although JOST reports that antheridia and oogonia were found on the same plant of *C. scutata*, such a condition has not been found on material used in the present investigation. Hundreds of disks were examined; but all, if bearing sexual organs at all, were found to be either antheridial or oogonial. So far as is known, all other species of *Coleochaete* produce both kinds of sex organs on the same plant, and it would not be surprising to find an occasional disk of this species doing the same.

The first antheridia formed are usually produced about halfway between the center and the circumference of the disk. According to PRINGSHEIM, a cell or a small group of cells begins division to form antheridia: first those adjacent to the dividing cells on a circular band whose center is the center of the disk divide, next those adjacent, and so on round the band. This continues until a band, more or less complete, extends around the disk. He reports the formation of other bands in the same thallus, all of which are formed in somewhat the same manner. My observations confirm his, save that instead of one group of dividing cells there may be several; and the band seems to be more interrupted than he indicates. Often sectors from one to four cells in width separate the bands into many groups. Scattered all through the groups there are cells that remain un-

changed. Often these are the cells that have borne hairs, but sometimes they are not. The band of antheridia seems to be wider and more irregular than PRINGSHEIM's description would indicate.

After the first band of antheridia has been formed, other bands arise. These may be either peripherally or centrally located with regard to the first band. A disk has been observed which showed a cell next within a peripheral one dividing to form antheridia (fig. 1 1*d*). Fig. 16 shows an antherozoid just ready to begin its escape. Only one cell separates the antheridium from the nearest peripheral cell. No peripheral cells have been found forming antheridia, and it is here that one finds a wide departure from the condition in other species of the genus, all of which form antheridia from terminal cells. In another thallus, a cell between the first band of antheridia formed and the peripheral cells had formed an antheridium before any other cell outside the first zone had gone through even the first division (fig. 4*a*). More often, however, an entire zone will have passed through the first division to form antheridia before the second division in the process of antheridial formation begins.

FIRST DIVISION.—The increase in both number and size of the cells of a disk is limited to the peripheral cells, as was noted by PRINGSHEIM, and as has been confirmed by other workers, including the writer (5). Once a cell ceases to be peripheral it never divides again, save for one of two reasons, so far as is known; for at just what time and for what reasons the thallus becomes two cells thick has not been determined by any of those who have studied the different species of this genus. Any cell in the interior of the disk may divide as the result of an injury to the adjacent cells, as previously reported (5); it may also undergo division in preparation for the formation of antheridia. This will hereafter be called the first division. The first wall formed in the latter case may be either radial or tangential. If either condition predominates it would seem to be the radial, although no attempt has been made to determine this point. A given sector may show only radial or only tangential divisions, although more often the two are indiscriminately mixed (figs. 6, 12). Fig. 2 1*d* shows a radial division which forms two rectangles from the outer surface wall; while fig. 3 shows the first wall dividing the outer wall of the cell into what in surface view appears as two nearly

equal triangles, reminding one of the divisions of the androgone mother cells in various Marchantiales. This first wall may (as it usually is) be formed at right angles to the plane of the outer wall of the dividing cell, or it may form an angle with the outer wall (figs. 7, 8). The angle thus formed may be so acute as to leave doubt as to whether the cell thus formed is an antheridium, or a cell resulting from the first division of a vegetative cell to form the two antheridia mother cells (fig. 7*d*). The two cells formed by the first division may thus be very unequal or they may be closely similar in shape and size (figs. 7, 9). If the thallus is two cells thick, the outer cell only is divided, the inner one, next the substratum, taking no part in antheridial formation (fig. 8). No case in which both had been divided has been observed.

SECOND DIVISION.—This division, which hereafter will be referred to as the second, which PRINGSHEIM thought to be the final one in antheridia formation, divides each of the daughter cells formed by the first division into a small antheridia and a large cell. The two second divisions may occur simultaneously, as shown in the lower cells of figs. 1 and 2, or they may occur at different times, as shown in fig. 4. Unlike the wall formed after the first division, the new partition wall always makes an acute angle with the surface plane of the two cells resulting from the so-called first division, as is shown by fig. 5. Although the angle is always acute its size varies, and the resulting antheridium also varies in size; this is why one cannot be sure whether the small cell formed by the division of the right hand cell in fig. 7 is an antheridium or one of the two daughter cells formed by the first division of the ordinary vegetative cell. The first antheridium of the pair formed by division of one of the two daughter cells is always larger than the second one formed from the same daughter cell. Fig. 5 shows that more than half of the upper portion of the cell is used.

THIRD DIVISION.—Soon after the second division, a third one occurs, which will hereafter be referred to as the third division. The large sister cells of the first antheridia formed from each of the two daughter cells of the first division divide to form another antheridium and a large sister cell. This third division follows so quickly after the second as to make it almost impossible to find one antheridium

cut from the two daughter cells of the first division without the second one. The third divisions may occur at the same time in the two daughter cells of the first division, or they may occur at different times, as in the case of the second division. This third division is followed by the formation of a wall that forms an acute angle with the plane of the outer surface of the cell undergoing division, as is the case after the second division. The opening of the angle, however, is in the opposite direction to that of the angle made by the wall that is formed after the second division with the plane of the outer wall of the original cell.

The second antheridium is slightly smaller than the first, as just noted; otherwise they are similar. An antheridium at the time of formation contains a very large nucleus, a small chloroplast, and more or less dense cytoplasm. The protoplast of each antheridium becomes a single antherozoid.

When the four antheridia formed thus from one cell are viewed from the surface, as PRINGSHEIM saw them, they give the effect of one cell divided to form four (fig. 2); but a section of the disk at right angles to the surface tells an entirely different story. This view even in section is not always the same, because it depends upon whether the section is cut parallel or at right angles to the first wall laid down. If the former, a large five-sided vegetative cell with two small antheridia which have been formed by division from one of the daughter cells of the first division is shown (fig. 6). If the section is at right angles to the first wall, there are two long narrow cells with one antheridium cut from the upper end of each (figs. 8, 13). Here the antheridial wall is usually at right angles to the lateral walls of the long narrow vegetative cells (fig. 13), but the antheridial wall may form any angle with the lateral walls (figs. 8, 9). Sometimes whole sections will have the antheridial walls at right angles to the lateral walls of the vegetative cells below; at other times they may all form acute angles with these walls; while still more frequently those forming right angles and those forming acute angles will be indiscriminately mixed (figs. 6, 12, 18).

VARIATIONS.—Each of the two daughter cells formed by the first division by a second and third division normally produces two antheridial cells. Sometimes more than two cells may be formed, how-

ever; that is, a fourth division of the sister cell to the second antheridium may result in a small cell similar in shape, size, and content to an antheridium (figs. 14, 15). Other similar divisions may occur, resulting in as many as five cells that resemble antheridia in every way. Whether all these antheridia-like cells function as antheridia has not been definitely determined, because, although the outer walls were ruptured and the contents gone, this condition might have been due to cutting. The size of the cells, however, their contents, and their position would lead to the conclusion that these small cells are all antheridia producing antherozoids as in the case of the first two usually cut off. None but cells producing antheridia are ever found to have formed these small antheridia-like cells. Although not abundant, they are by no means infrequently found. Certainly this species is quite variable in all other respects, therefore it may well vary in this, forming several antheridia from each daughter cell of the first division.

SISTER CELL.—At the third division the sister cell of the second antheridium usually does not undergo further cell division, although in a few cases division must have occurred either after the formation of the first antheridium or at least at some time after the first division. At any rate, where one cell is usually, occasionally two cells are found. This sister cell of the last antheridium formed is a seven-sided vegetative cell. The outer wall, the one opposite the substratum, is made up of two walls, of which a section shows a cell with five walls (figs. 6, 7). In case the thallus is two cells thick, two vegetative cells occupy the space usually occupied by the one sister cell of the last antheridium formed. These cells have the usual contents; each possesses a nucleus, cytoplasm, and a chloroplast (figs. 6–9).

CHLOROPLAST.—All writers on this species agree that each antherozoid (only one being formed in each antheridium) is green in color, while those of the other species of this genus are colorless. Never having seen the swimming antherozoid, it is impossible for me to confirm this statement. The evidence from surface views and from sections is conflicting. From the surface view (fig. 2*an*) no chloroplast could be seen. Sections through antheridia soon after their formation showed a definite but small plastid in each. Other an-

theridia showed plastids that seemed to be disintegrating. These were smaller, showed no pyrenoid, were very vacuolate, and took the stain very lightly. Others showed no sign of a chloroplast. This might have been a result of cutting, but the very large nucleus was clearly visible in each case, and all zoospores showed the chloroplast closely associated with the nucleus. Numerous antherozoids which were escaping from the thallus were examined and not one showed a chloroplast (figs. 16, 17). Zoospores at this stage never fail to show chloroplasts. If the antherozoid has a chloroplast, what becomes of it? Zygotes whose chloroplasts have just begun to divide showed no sign of the presence of a chloroplast from the antherozoid.

ANTHEROZOID ESCAPE.—The antherozoid escapes through a pore that, from all indications, is formed in the same way as that for the escape of the zoospore. An opening is formed for the escape of each zoospore, but the two antherozoids formed in the antheridium that has been formed by the division of one of the daughter cells of the first division and in the antheridium that has been formed by the division of this sister cell of the first antheridium may escape through the same pore (figs. 7, 10). Sometimes, however, the two antherozoids formed in the manner just described may escape through different pores (fig. 12). The manner of escape corresponds exactly to that of the zoospores (figs. 16, 17) as previously described (5). That the male gametes escape in great numbers is shown by figs. 18 and 20. In section the fragments of the antheridial walls can be clearly seen but in surface view the thallus has a decidedly ragged appearance due to the presence of fragments of the old antheridial walls.

SISTER CELL CHANGES.—At first the outer walls of the sister cells form an acute angle (figs. 7, 12). Soon after escape of the antherozoids, the acute angle is retained and the walls are thin and delicate. Later these outer walls become gradually thicker, until they are approximately as thick as the usual surface wall of a vegetative cell. Soon the pressure of the contents eliminates the sharp angle of these two outer walls, and the two become rounded and thereafter have the appearance of one wall rather than two. These sister cells of the antheridia mother cells may form hairs like any other young cell. Sometimes these hairs are found projecting through the pores

through which the antherozoids have escaped (figs. 19, 20). In a thallus two cells in thickness, the inner cell may likewise form a hair after the escape of the zoospore from the outer cell (5).

Discussion

Although the manner of formation of antherozoids differs widely in the various members of the Chlorophyceae, none described for other genera in any way approximates the method found in *Coleochaete scutata*. The Chlorophyceae range from the Conjugatae in which the apparently unchanged content of an ordinary vegetative cell acts as a male gamete, to the Charales which have the most complex antheridia found in any plant. Between these two extremes appear several gradations in antheridium formation. In *Ulothrix*, for example, the content of a vegetative cell divides to form a number of gametes which may or may not be differentiated into male and female. In *Vaucheria* a special cell, an antheridium, is developed for the production of antherozoids, and produces many. *Oedogonium* may have its antheridia on a male plant exactly like the female plant, on a small, few-celled plant epiphytic on the female, or on the same plant with the oogonia. In every case the antheridium is a cell exactly like the ordinary vegetative one save that it is much shorter. It is produced in exactly the same way as is the vegetative cell. In each antheridium two antherozoids are produced, or sometimes only one.

In all species of *Coleochaete*, so far as known, the antherozoids are produced in special cells. In *C. pulvinata*, *C. irregularis*, and *C. nitellarum* the antheridia develop from the terminal growing cell. These cells, the antheridia, are produced just like any vegetative cell except that they have no chloroplast, are smaller, and more pointed at the outer end. The antheridia occur in groups. One colorless antherozoid is produced from each antheridium. This method is best illustrated in *C. pulvinata* (2).

Unlike these, *C. scutata* produces its antheridia by division of an interior cell of the thallus which ordinarily would not divide again. There is no increase in size, no protusion of the cell wall, nor any other noticeable change unless the protoplasm becomes slightly more

dense. The nucleus of each daughter cell of the first division divides following the unequal division of the chloroplast; and a second cell division forms another antheridium. In each, one antherozoid is formed. The two antherozoids usually escape through a common pore, which is apparently formed by enzymatic action. This method of antheridium production seems to be unique, and would seem to place *C. scutata* next to the Charales in the complexity of the development of the antheridium.

Summary

1. Antheridia are produced from the middle of June until the middle of October in the region in and about Chicago.

2. The cells from which bands of antheridia are formed may begin by the division of a single cell or by division of groups of cells some distance apart. The first group arises about halfway between the center and the circumference of the disk. Outer and inner bands appear later, sometimes arising almost simultaneously.

3. The first division of a vegetative cell produces two daughter cells, antheridia mother cells, by whose division antheridia are formed.

4. The second division produces an antheridium from each daughter cell formed at the first division.

5. The sister cell of the second antheridium is a large seven-sided vegetative cell, which remains after the escape of the two antherozoids from the antheridia.

6. More than four antheridia may be developed from one vegetative cell.

7. The outer wall of the sister cell of the last antheridium formed becomes rounded and thickened, until it takes on more or less the appearance of the outer wall of the ordinary vegetative cell.

8. Hairs are sometimes produced by these sister cells of the last antheridium formed.

9. Some antheridia contain normal chloroplasts, some contain chloroplasts that appear to be disintegrating, while still others show no chloroplasts at all.

10. The antherozoids formed from each of the daughter cells of the first division usually escape through a common pore.

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AMARILLO
TEXAS

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EXPLANATION OF PLATES V, VI

All figures were drawn with the aid of the camera lucida on a level with the base of the microscope. Spencer apochromatic objective 1.5 mm. N.A. 1.30 was used. Compensating ocular 15× was used for figs. 1–4, 16, and 17, giving a magnification of 1370×; a Zeiss 4× compensating ocular was used for all other figures, giving a magnification of 1020×. All figures are reduced one-half.

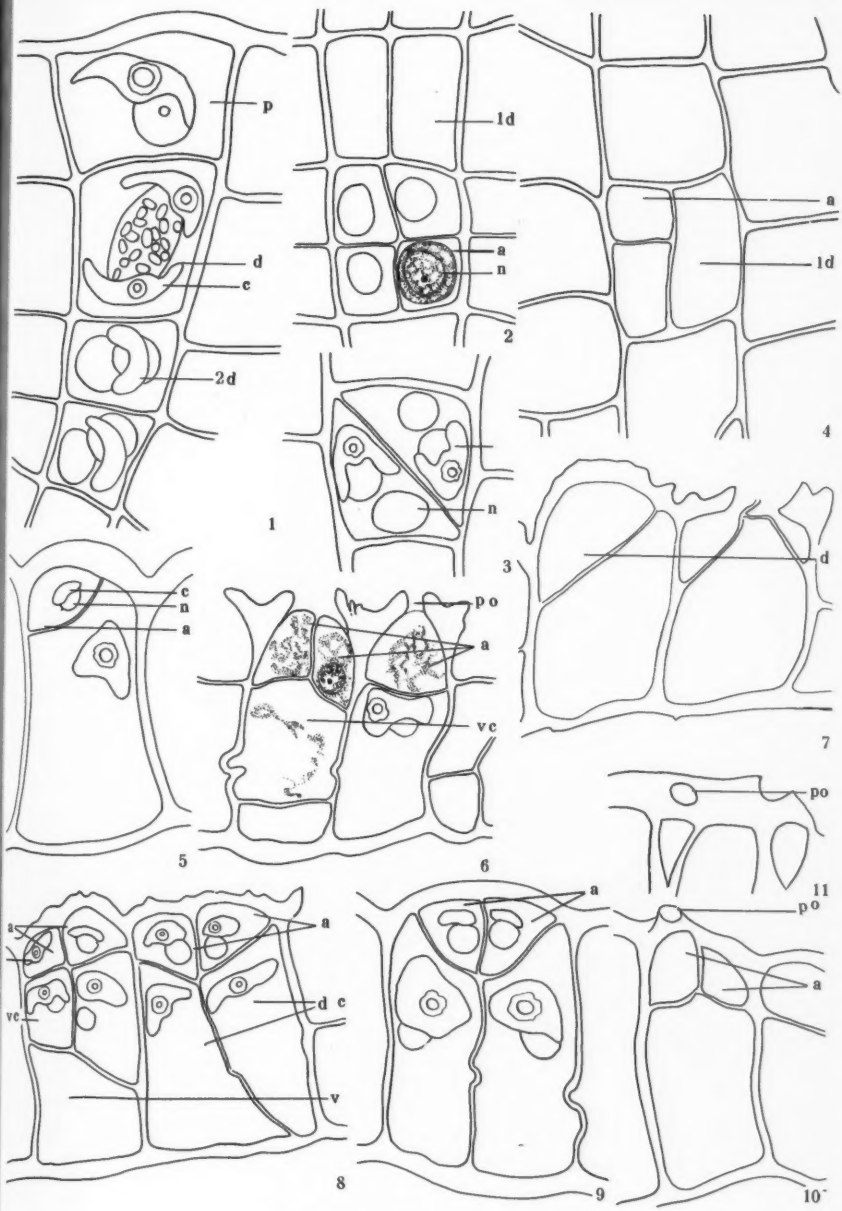
The small letters on all plates have the following meanings: *a*, antheridium; *an*, antherozoid; *c*, chloroplast; *cy*, cytoplasm; *1d*, first division; *2d*, second division; *3d*, third division; *g*, granules; *n*, nucleus; *p*, peripheral cell; *po*, pore; *vc*, vegetative cell.

PLATE V

FIG. 1.—Surface view of peripheral cell, next cell in late prophase for first division to form antheridium, and third cell in from periphery showing nucleus divided but wall not yet formed.

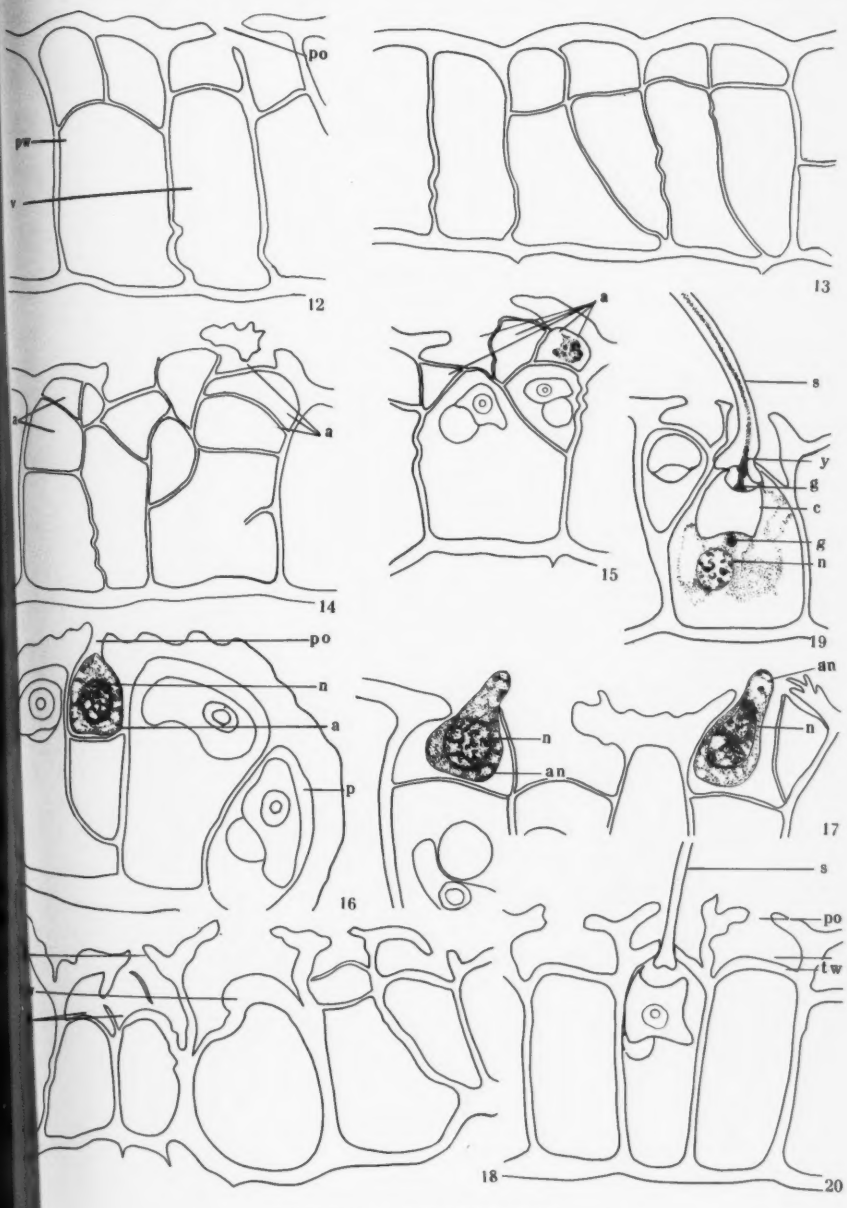
FIG. 2.—Surface view of two cells, upper showing first division, lower showing completed antheridia.

FIG. 3.—Surface view of cell showing wall formed at first division passing diagonally across cell.



WESLEY on COLEOCHAETE

BOTAN
pw
v
a



WESLEY on COLEOCHAETE

FIG. 4.—Surface view of cell near outer edge of disk, showing at least one antheridium while no other cell outside the first band formed has even undergone first division.

FIG. 5.—Section through thallus at right angle to surface and parallel to first wall, showing first antheridium formed.

FIG. 6.—Section similar to that shown in fig. 5, showing second antheridium formed by division of sister cell of first antheridium.

FIG. 7.—Section showing cell at left which cannot be definitely placed as either one of the two daughter cells or as first antheridium; cell at right shows two empty antheridia with common pore and remaining vegetative cell.

FIG. 8.—Section showing in cell to left that only upper of the two cells is involved in formation of antheridia (section cut parallel to first wall formed); in this and cell to right may be seen variations in angles made by antheridial walls with lateral walls of adjacent vegetative cells; cell to right shows inequality of two cells formed by first division.

FIG. 9.—Wider variation of walls separating antheridia and vegetative cells.

FIG. 10.—Section through thallus cut just to side of pore.

FIG. 11.—Section through thallus cut at angle to surface giving small portion of surface in which pore was located; just below is empty antheridium.

PLATE VI

FIG. 12.—Section through thallus showing cell at left cut parallel to first wall, while one at right is cut at right angles to first wall; one at right shows common pore for two antheridia which have had their origin in different daughter cells.

FIG. 13.—Section showing number of cells cut at right angles to first wall; some show antheridia formed from them while others do not.

FIGS. 14, 15.—Sections of cells showing more than two antheridial-like cells formed from one daughter cell and its descendants.

FIG. 16.—Section of thallus showing antherozoid ready to escape before third division has taken place; only one cell between it and peripheral cell.

FIG. 17.—Section showing two antherozoids passing out of pores.

FIGS. 18, 20.—Sections through thalli after escape of antherozoids, showing remains of old antheridial walls and thickened outer wall of remaining vegetative cells.

FIGS. 19, 20.—Sections showing hairs formed by vegetative cells left after escape of antherozoids; fig. 19 shows section through hair while fig. 20 shows section cut just to side of hair.

FLOWER PRODUCTION BY ORCHID GROWN NON-SYMBIOTICALLY

LEWIS KNUDSON

(WITH THREE FIGURES)

Introduction

In a number of papers previously published (**6–9**), issue has been taken with the view advanced by BERNARD (**1**), BURGEFF (**3**), and others that the orchid fungus is necessary for germination. In these various papers evidence was presented demonstrating that the germination of orchid seeds is dependent on an outside source of organic food. If a utilizable sugar is provided germination proceeds readily; if starch is provided there is no germination unless some means is provided for converting the starch into sugar. This may be accomplished by supplying the orchid fungus, or by the use of other fungi which will digest the starch and provide an appropriate hydrogen-ion concentration without at the same time growing so luxuriantly as to prevent growth of the orchid embryos. In the earlier papers the view was emphasized that the action of the orchid fungus is purely external, and therefore of no particular significance to the orchid embryo unless starch is available or other digestible food is supplied.

It is not necessary here to summarize the evidence for the non-symbiotic view. A recent paper (**9**) discussed the evidence for and against obligative symbiosis. The present paper is concerned primarily with the view held by some that obligative symbiosis is a requisite for flowering of the orchid plant.

In a review of my first paper, COSTANTIN and MAGROU (**5**) intimated that seedlings produced by the non-symbiotic method would not flower. COSTANTIN (**4**) referred to this in a second paper, but later BULTEL (**2**) showed that plants produced from seedlings germinated asymbiotically would flower; and my results (**9**) were confirmatory. In the development of the orchid plants to the flowering stage, infection of the roots occurred both with BULTEL's plants as

well as mine. Referring to BULTEL'S work, COSTANTIN (4) intimates again that the flowering of these plants was due to infection of the roots by the orchid fungus, and states that the fungus probably contributes a vitamine which is necessary for reproduction. In a review of my second paper COSTANTIN (4) invites me to produce flowers on plants raised asymbiotically, with the intimation that this is not possible.

It was hoped that seedlings transplanted and grown in osmunda fiber would develop without infection, since the plants were maintained in a greenhouse not previously used for orchids. MERCIER'S (10) work showing the wide distribution of the orchid fungi showed clearly the improbability of maintaining orchid plants free of the fungus when the plants are grown in the open. Fortunately another experiment had been devised to test the theory proposed by COSTANTIN that an orchid plant would not flower without fungus infection. In this particular experiment an attempt was made to develop an orchid plant to the flowering stage under pure culture conditions.

Experimental method

Seeds of a *Laelia-Cattleya* hybrid were sown in a tube culture, using the methods previously described (6). The culture solution used was Pfeffer's, the phosphate supplied being monobasic potassium phosphate. To this solution was added 2 per cent glucose. The agar content was 1.5 per cent and the reaction of the sterilized culture medium was pH 6. The initial sowing was made on October 20, 1920. Owing to the high pH value growth was relatively slow, and the seedlings were left in the tube until August 4, 1921. By this time the culture medium was reduced to about one-half of the original volume, and the seedlings had developed two small leaves with the first root just beginning to appear. On August 4, 1921, a few seedlings were transferred to a 250 cc. Erlenmeyer flask containing solution B with 1.5 per cent agar but without any sugar. The pH of this solution was 5.8. Growth on this medium was again relatively slow, and the leaves were yellowish green, due to the low availability of iron. On December 22, 1923, a single plant with several leaves 2 inches in height was transferred carefully to a 12-liter flask containing 4 liters of solution B of one-half concentration, and

adjusted by the addition of N/10 hydrochloric acid to a pH value of 4.9. The agar content was 1.5 per cent. Previous experiments had revealed this as the most favorable value with solution B. It may be mentioned that this pH value permits the availability of iron by maintaining it in solution. It should be added that sugar was not added to the culture medium.

The 12-liter flask with the culture medium was sterilized by autoclaving, and in transplanting the seedling every precaution was taken to insure sterile conditions. After transplanting, the flask was taken to the greenhouse where it was placed in a shaded chamber to prevent any burning of the inclosed plant by the sun.

Results

New growth quickly appeared, and after 8 months in the large flask the plant had the appearance shown in fig. 1. From December, 1923 to June, 1927 the culture remained free of any organisms; thereafter for some reason not apparent certain contaminations appeared. The first apparent contamination was an alga, a species of *Chlorella*, and later appeared a small colony of a moss and a contamination resembling *Penicillium*. The orchid plant continued to thrive until late in 1928, developing well (fig. 2), with a very abundant root system. The roots for the most part penetrated the agar medium and some extended to the bottom of the flask. During the entire period (December, 1923 to November, 1928) no additions were made to the culture medium, and not more than 2 liters of water had evaporated at the end of this time.

Concurrently with the transfer of a plant from the Erlenmeyer flask to the large one, about twenty-five seedlings were transplanted to pots containing osmunda fiber, as is usual in practical procedure. These plants developed in a manner comparable with the one in the flask. Of the initial seedlings transplanted in pots twenty survived. Of these, five flowered from October to December, 1927; the remaining ones flowered in October, November, and December, 1928. The roots of five of these plants were examined in 1927 and 1928 and the fungus was found to be present.

The single plant in the large flask finally flowered in November, 1928 (figs. 2, 3). The flower stalk produced two flowers which were

normal in every respect. The flower stalk, however, was less rigid than is usual for plants grown in the open. This no doubt was due partly to the high humidity prevailing in the flask and partly to the shaded condition under which the plant developed. The flower not

FIG. 1.—Plant 3½ years old; reduced to one-third

only structurally but otherwise was similar to the flowers produced in the open on plants with infected roots. Unfortunately, before photographing the plant the flask was left for three days in the laboratory, and either because of fumes or the dry atmosphere, the petals began to droop just before the photograph was made.

In regard to other features, perhaps the outstanding one was the

very extensive development of the root system (fig. 3). Of a total of twenty-seven main roots produced only two had died. The others as well as the lateral roots were in a healthy condition. As would be expected, the roots were all slightly green or greenish in color, and microscopic observation revealed the presence of chloroplasts in

FIG. 2.—Plant flowering in November, 1928; reduced to one-fourth

every root. The viability of these roots is in striking contrast to that of roots growing in osmunda fiber in pots.

At the conclusion of the experiment the flask was broken so that the culture medium could be examined and the plant removed for further observation. Immediately ten small samples of the agar medium were removed from different areas at the surface of the agar and platings made. Similarly eight samples of agar were re-

moved from the agar mass at a depth of about 2 cm. from the surface. A potato agar medium was used as the culture medium for plating. The petri dishes were incubated at 26° C., and there developed in the

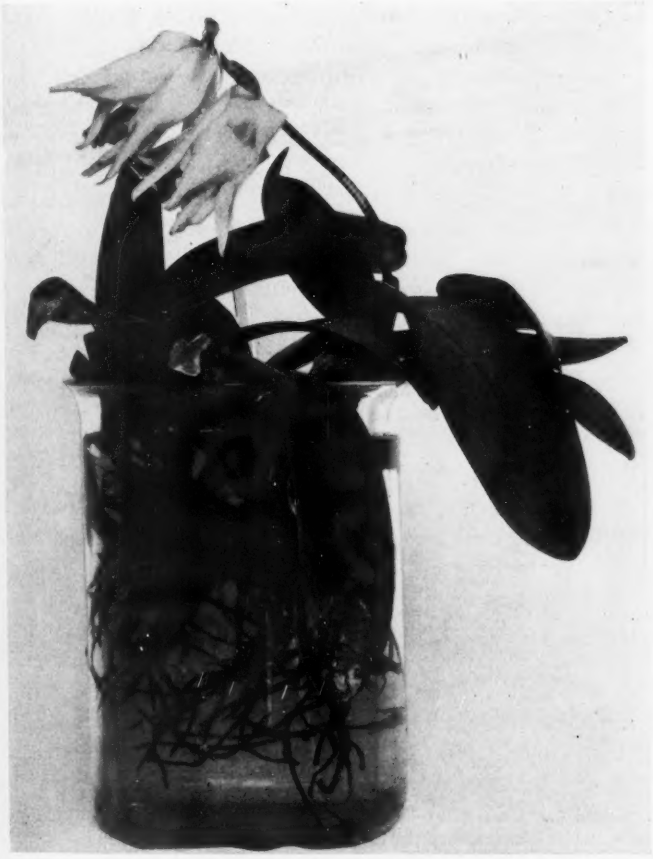


FIG. 3.—Details of plant removed from flask

lot two forms of *Penicillium*, one *Fusarium*, and two forms of yeast with several bacterial colonies apparent. There was no evidence of the presence of the orchid fungus.

Microscopic examination of the culture medium was made immediately following the removal of the plants. The apparent contaminations were a species of *Chlorella* which practically covered the surface. There was in addition a colony of moss. There was noted also some fungus growth but none of the hyphae observed had the characteristic appearance of the orchid fungus.

To establish the absence or presence of the fungus organism in the roots, freehand sections were made of every main and secondary root of the orchid plant. For the main roots, sections were made about 0.5 cm. behind the tip and at regular intervals up to the base. Three or four regions were examined from every main root, and in like manner three regions were examined in every secondary root. A total of sixty-five roots was examined. Four or five sections were made in each case and every one carefully examined. In no case was there any evidence of infection. As stated previously, all of the roots were green or slightly green in color, and the cortical cells of all roots had chloroplasts. This is of some significance as it has been the general observation that roots containing chloroplasts are not invaded by the orchid fungus.

Conclusion

A consideration of the evidence here presented must lead to the conclusion that the symbiotic condition is not obligative either for germination or for growth and flower production. The contention may be made that the plant was grown under purely artificial conditions with a relatively high concentration of nutrients, and that organic matter may have been supplied because of the contaminations. It is true of course that the seedling was grown for 10 months in the presence of sugar, and COSTANTIN has several times stated that the high concentration of sugar in its effect is similar to those chemical substances effective in artificial fertilization. This argument has been adequately answered in a previous paper (9), however, and needs no further consideration. The contention may be made also that organic matter may have been available to the orchid plant as a result of the contamination that occurred during the last 18 months of growth. The hydrolysis of some of the agar would yield galactose, but unpublished data show that this sugar is not utilizable by the orchid plants. Some sugar may have resulted from

the decomposition of *Chlorella*, but the amount would have been very slight or the growth of *Penicillium* and *Fusarium* would have been markedly increased and not just visible, as was the case. It may be concluded that no more organic matter was available for the orchid grown in the flask than is available for epiphytic orchids growing under natural conditions, where decay of moss, lichens, and bark is constantly in progress. In conclusion it may again be stated that obligative symbiosis is not requisite either for germination or for flower production.

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OXIDATION-REDUCTION INDICATORS AS A MEANS OF DETERMINING OVERHEATING IN WALNUTS DURING DEHYDRATION[1]

A. R. C. HAAS

Among the chief advantages of dehydrating walnuts by artificial heat over sun-drying are the improvement of quality and the early marketing because of rapid curing. As found by BATCHELOR and others (1), the highest temperature at which walnuts can be dehydrated with safety is 110° F., although with very moist nuts 105° F. was found to be safer. To reduce the time and cost of dehydration, the nuts are dried at a temperature as close as possible to the so-called "critical" temperature beyond which they cannot be heated without danger of injury. Since dehydrators are sometimes operated by growers who do not realize the consequences of overheating, the product in some cases becomes rancid by the time it reaches the consumer. This involves not only a financial loss, of course, but also a loss of reputation to the marketing association. If a method could be found by which overheated nuts might be detected before they are mixed with other nuts, the annoyance and financial loss due to overheating would be reduced to a minimum.

In this paper a method is described which the writer believes is reliable and practical. Preliminary tests gave indications that some method based on oxidation and reduction would be a most promising one. While these studies were under way an article by HIBBARD and MILLER (3) appeared in which it was pointed out that seeds of varying degrees of viability reduce very dilute solutions of potassium permanganate. The indicator, however, proved to be too rapidly reduced by walnut material to be of use in the present investigation, but tests with other oxidation-reduction indicators confirmed the claim of HIBBARD and MILLER that the underlying principle involved is oxidation-reduction.

In the tests to be described, the oxidation-reduction indicators developed by CLARK and others (2) furnish the basis for the method.

[1] Paper no. 141, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The principles involved are discussed by TAYLOR (6) and NEEDHAM and NEEDHAM (5).

The method briefly is as follows. Walnut kernels from two sources (control nuts used as a standard, and the nuts suspected of being overheated) were finely mashed and mixed in mortars. Samples of equal weight were weighed and placed on small pieces of paper. The samples in each case were added to test-tubes containing a known amount of buffer solution (about pH 7) containing the oxidation-reduction indicator. After an arbitrarily chosen period the tubes were shaken and the contents filtered. The colors of the solutions in corresponding tubes were compared and the sample showing the greater degree of reduction considered overheated with respect to the sample chosen as standard.

In the method used by the writer six walnuts were selected from a lot known to have been dehydrated at 110° F. for 24 hours. This standard was chosen arbitrarily as no studies were undertaken to establish a fixed standard applicable to practice. Six walnuts were also selected from the lot of nuts supposedly overheated. Only those nuts were selected which were well sealed, the kernel free from shrivel, mold, etc., and as light in color as the lot would afford.

The contents of the selected nuts in each case were finely mashed and mixed in porcelain mortars. The use of aqueous extracts of weighed samples as reducing agents was found to be unsatisfactory. Ten 2-gm. samples of mash from each of the two lots were quickly but roughly weighed out and placed on individual papers. This permitted duplicate determinations by using five oxidation-reduction indicators. These samples were quickly transferred to test-tubes containing 15 cc. of solution plus an oxidation-reduction indicator.

The solution employed was a modified HOAGLAND'S solution (4), in which the phosphate was supplied as the di-potassium hydrogen phosphate instead of the mono-potassium di-hydrogen phosphate, and in which iron and manganese were omitted. Such a solution has a pH of 6.8–7, and is considerably buffered. The temperature of the room in which the determinations were made was close to 30° C. The solution was first poured out into beakers; sufficient indicator was then added to give a deep color; and after being stirred, 15 cc. of the solution was run into each of four test-tubes previously graduated to

15 cc. Another similar set of ungraduated test-tubes, but of uniform diameter and color, were reserved for the final color comparisons.

Of the oxidation-reduction indicators that were tried (6), some were found (table I) to be reduced too easily to be of value in the present tests. The indigo sulphonates were the most satisfactory of the group designated as excellent in table I.

TABLE I

RELATIVE POSITIONS OF OXIDATION-REDUCTION INDICATORS

INDICATOR	POTENTIAL IN VOLTS AT pH 7 AND 30° C. (6)
Too easily reduced	
2- Chloro-indophenol (o-chlorophenol indophenol)	+0.233
Indophenol (phenol indophenol)	+0.228
2- Methyl-indophenol (o-cresol indophenol)	+0.195
Excellent	
1- Naphthol 2-sulphonate indophenol	+0.123
Methylene blue	+0.011
Indigo-tetrasulphonate	−0.046
Indigo-disulphonate	−0.125
Indigo-monosulphonate	−0.156

The procedure of the tests was as follows. The weighed samples of mashed walnut kernels were in each case rapidly transferred from the papers to the test-tubes containing the measured amount of solution plus indicator, and thoroughly shaken under comparable conditions. The tubes were left standing from 15 to 30 minutes until the reduction was well under way. With little practice one can learn the concentration of indicator required to bring about the most contrast in color at the end of the period of standing. When the tubes had stood the desired period, the comparable tubes were shaken at the same time and at once filtered into the test-tubes already prepared. A good grade of filter paper (Whatman no. 40) was used, since it was found that ordinary paper permits finely divided particles to pass through into the solution, giving an opalescence that interferes considerably with the color comparisons.

A 200 watt 120 volt nitrogen-filled lamp served as a source of light, in front of which was placed a piece of Dalite glass. The color comparisons were made against a porcelain plate background, using reflected light. Although it is desirable to compare the time required

for equal reductions, a comparison of the degree of reduction in equal time appeared to be the most practical method and gave consistent results.

Many electric ovens, especially when in use for some time, give temperatures that may be far from uniform. In preparing heated nuts for experimental purposes, therefore, maximum and minimum thermometers as well as other accurate thermometers were placed in the oven on the same level as the nuts to insure accurate temperature control. In all of the tests the walnut varieties were mixed indiscriminately, so that varietal differences are not of much importance. Taking walnuts heated at 110° F. for 24 hours as a standard, and a sample heated to 120° F. or above, it was found to be a relatively simple matter to determine which of the two had been "overheated." About twenty-five samples of walnuts known to have been overheated during dehydration at dehydration plants or in the laboratory were all found to show a greater degree of indicator reduction in a given time than the control nuts. Moreover these tests were run within a few days after the nuts were dehydrated.[2] When such samples were stored for a month or more, the tests were repeated with the same results but somewhat more pronounced. It was possible with the aid of the adopted standard to detect nuts heated supposedly at 110° F. for 24 hours but during which time the temperature had been at 117°, 126°, or 130° F. respectively for a few hours. Nuts heated at 120° for 18 hours showed greater reducing action than nuts heated at 110° for 24 hours. Twelve-hour heating of nuts at 100°–103° F. gave less reduction than 24-hour heating at the same temperature, and the former showed "overheating" in comparison with a sample of the unheated nuts.

A lot of nuts that had been subject to air-drying in storage for about two months were divided into samples of several hundred nuts each. Of samples heated for 24 hours, those at 120° showed overheating with respect to those heated at 110° F. When samples were heated to 120° for 12 hours they still showed overheating.

With respect to the standard samples heated at 110° F. for 24 hours, heating at 120° for 24 hours showed slightly greater reducing action than heating at 112°–114° for 24 hours. It is possible therefore

[2] The writer appreciates the assistance given in some of the preliminary routine by Mr. O. L. BRAUCHER, Field Investigator of the California Walnut Growers' Association.

to study by such a method the equivalent effects of heating. As the "overheating" becomes excessive the reducing action becomes the more rapid, and when "overheating" is doubtful the differences in color with the several indicators will usually be very small if not indistinguishable or inconsistent. When all of the indicators show a decidedly more rapid reduction in the one case than in the other, it is practically certain that "overheating" has taken place in the former sample.

Summary

1. A simple and delicate method is described in which oxidation-reduction indicators are used in buffers of approximate neutrality for the detection of "overheating" in walnuts.

2. Mashed walnut kernels show a strong reducing power which is increased when the nuts are "overheated."

3. It is possible by the method described to study equivalent effects of heating when the temperature and time of heating are varied.

4. "Overheating" is quite as satisfactorily detected in freshly dehydrated nuts as in those held for some time in storage.

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CURRENT LITERATURE

BOOK REVIEWS

Gnetales

In 1904, soon after his appointment as Bolus Professor of Botany in the South African College at Cape Town, Professor PEARSON visited the Damaraland Desert and made a thorough field study of *Welwitschia*, which at that season was in full bloom. He took photographs and collected material for a morphological investigation. During subsequent trips he traced the change in the flora from Kalahari Plateau into the desert. As he worked upon the morphological material, he realized the need for a comparison with *Gnetum*, and accordingly visited Angola, where he made field studies and collected material. As his papers began to appear, he was prevailed upon to write a general account as a volume in the series of Cambridge Botanical Handbooks. Much of the book was written before he died, in November, 1916. Professor SEWARD took the unfinished manuscript and, with the assistance of Mrs. THODAY, who had written several papers on *Ephedra* and *Welwitschia*, completed the work.[1] The last chapter, in its present form, is based upon PEARSON'S notes and upon two papers which were published after his death.

There is a preface by the editor, A. C. SEWARD, and five chapters: (I) Habit, distribution, ecology, taxonomy; (II) Vegetative morphology and anatomy; (III) The inflorescence and flower; (IV) Reproduction; (V) Theoretical chapter. There is also an extensive bibliography and index.

Throughout the book the treatment shows a wide knowledge of Gnetales in the field; and the author's thorough training in taxonomy increased the value of the ecological descriptions.

So few botanists have studied *Welwitschia* in the field that it is worth while to note a few points. Large plants attain, at the top, a diameter of 4 feet, but seldom extend more than 18 inches above the surface. Often they are nearly covered with sand, which frequently gets into the depression in the top, and in moist seasons supports a growth of epiphytic grasses and other plants. Individuals growing close together may fuse into irregular complexes. The young leaves are erect but soon become depressed, so that the distal ends rest upon the ground and become torn to ribbons by the wind. The plant produces only two leaves, which are constantly renewed at the base but which seldom reach more than 6 feet in length because they die off at the distal end. The roots are remarkably long, penetrating to moist soil. The dry habitat may not be necessary,

[1] PEARSON, H. H. W., Gnetales. Large 8vo. pp. vi+194. *pls. 3 and portrait. figs.* 90. Cambridge University Press. 1929.

or even desirable, for plants grown in the greenhouse thrive better in the orchid room than in the room for desert plants.

The range of *Welwitschia* from north to south is about 700 miles, from the mouth of the San Nicolan River in latitude 14° S. to a locality along the Rio Caroca in latitude 15°50′ S. Plants extend from the sea coast inland for about 20 miles; at Walvis Bay about 30 miles. In parts of this range the average yearly rainfall is only 1 inch. The classic station, where the plant was discovered by WELWITSCH, is near Cape Negro, on a plateau about 400 feet in height. When PEARSON visited this locality in April, 1909, after a period of exceptionally heavy rainfall, a luxuriant growth of *Aristida* made the region look like a cornfield, so that *Welwitschia* was hidden by the tall grass. Seedlings were numerous. It is probable that seeds retain their vitality for several years and produce abundant seedlings in such seasons.

In treating the vegetative anatomy, special attention is given to seedlings, and conditions in the three genera are compared. In discussing the "perianth" of *Welwitschia*, the conclusion is reached that the mere fact that it is vascularized is not sufficient to establish the claim that it is a foliar structure: just what it may be is still to be proved. If the expanded inner integument of the male flower represents an abortive stigma, why is it retained in the male flower, where it does not function, but lost in the female flower, where it might have functioned? Although the other parts of the flower are so diverse in the three genera, the ovules are very similar. A series of diagrams emphasizes the similarities.

The reduction in the female gametophytes is traced, and the three genera are compared, the series being *Ephedra*, *Welwitschia*, *Gnetum*, with *Gnetum* as the most reduced form, having nearly reached the angiosperm condition. So far as the extent of development is concerned, the female gamete in *Welwitschia* is formed in the twelfth or thirteenth division from the megaspore mother cell; in *Gnetum*, somewhat earlier; and in angiosperms, still earlier. Fusions of nuclei in the female gametophyte, stimulating the development of endosperm, are compared with the triple fusion in the double fertilization of angiosperms.

The interrelationships of the three genera are still obscure, and there is no proof of any near affinity; but there is reason to believe that they are the existing remnants of an ancient race which was once more numerously represented. Perhaps the ancestors of the angiosperms were not far removed from *Gnetum*. In anatomy, and in the female gametophyte, the resemblance is to conifers.

The dichasial cymes of Gnetales are not found in recent gymnosperms, but they occur in *Wielandiella*. The resemblance between the long micropylar tubes of *Gnetum* and the Bennettitales, and the shape of the integuments have been emphasized by THODAY and BERRIDGE; but these comparisons afford no proof of phylogenetic relationship.

The general conclusion is that relationships of the three genera of the Gnetales are as obscure now as they have been at any time within the past decade; but the evidence indicates that, in spite of the occurrence in them of some angiosperm characters, they are essentially gymnosperms.—C. J. CHAMBERLAIN.

Belgian coal ball studies

Miss LECLERCQ has continued her initial studies of Belgian coal balls[2] by a series of equally interesting and valuable notes.[3] The text in all these publications is supplemented by excellent illustrations, which are based upon photographs accompanied by ample legends.

It is a rich flora which is presented. It belongs to the Lower Carboniferous and to the Lower and Middle sections of the Upper Carboniferous. These last two sections of the Upper Carboniferous should be numbered in accordance with the recently established classification of the European coal measures, Westphalian *A* and *B*, and correspond to our Pottsville.

The following species are described: *Calamites communis* Binn., *C. binneyana* Schim., *C. casheana* Will., *C.* sp., *Sphenophyllum plurifoliatum* Will., *S. gilkineti* Suz. Leclercq., *S. dawsoni* Will., *Lepidodendron selaginoides* Carr., *L. harcourti* With., *Lepidophloios fuliginosus* Will., *Lepidostrobus fuliginosus* Will., *Lepidocarpon* sp., *Bothrodendron mundum* Will., *Sigillaria* sp., *Stigmaria ficoides* Sternb., *S. lohesti* Suz. Leclercq., *S. bacupensis* Will., *S. weissiana* Leclercq, *Etapteris scotti* P. Bertrand, *E. lacatei* Ren., *Ankropteris bibractensis* var. *westphaliensis* P. Bertrand, *A. corrugata* (*stipe*) Will., *Stauropteris oldhamia* Binn., *Botryopteris ramosa* Will., *B. cylindrica* Will., *Psaronius* sp., *Lyginopteris* sp., *Cordaites felicis* Benson, *C. weristeri alpha* Leclercq, *C. weristeri beta* Leclercq, *Amyleon radicans* Will., *Mesoxylon* sp., *Trigonocarpus* sp.

The Belgian coal balls offer a rich field for the study of the structure of carboniferous plants, and it is to be hoped that Miss LECLERCQ will continue to publish contributions from this source.—A. C. NOÉ.

Protoplasm

An international series of monographs[4] on protoplasm has recently been started under the editorship of F. WEBER (Graz) and L. V. HEILBRUNN (Woods Hole), with the collaboration of R. CHAMBERS (New York), E. FAURÉ-FREMIET (Paris), H. FREUNDLICH (Berlin), E. KÜSTER (Giessen), F. E. LLOYD (Montreal), H. SCHADE (Kiel), W. SEIFRIZ (Philadelphia), J. SPEK (Heidelberg), and W. STILES (Reading).

Volume III in this series deals with the pathology of the plant cell,[5] and its first part is devoted to the pathology of protoplasm, for which it intends to be an introduction rather than a monograph.

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[2] LECLERCQ, SUZANNA, Introduction à l'étude anatomique des végétaux houillers de Belgique: Les Coal balls de la couche Bouxharmont des Charbonnages de Wérister. 4vo. pp. 79. *pls. 49.* Mém. Soc. Géol. Belgique. Liége. 1925.

[3] ———, Les Végétaux à structure conservée du houiller Belge. Note 1. 8vo. pp. 14. *figs. 6.* Note 2. pp. 7. *figs. 6.* Note 3. pp. 14. *figs. 7.* Note 4. pp. 7. Ann. Soc. Géol. Belgique t. LI. Bulletin. Liége. 1928.

[4] Protoplasma-Monographien. Berlin: Gebrüder Borntraeger. 1928.

[5] KÜSTER, E., Pathologie der Pflanzenzelle. Teil I: Pathologie des Protoplasmas. pp. viii+200. *figs. 36.* Berlin: Gebrüder Borntraeger. 1929.

The book is divided into two chapters: changes of form (Formwechsel), and structural changes (Strukturwechsel). The first chapter is subdivided into the following sections: (1) plasmolysis; (2) artificial deformation; (3) division protoplasts; (4) dislocation of protoplasm; (5) protomoptysis and related phenomena; (6) local necrosis; and (7) increase in volume of denuded protoplasts by swelling.

The second chapter contains such sections as (1) changes in the stratification of protoplasm; (2) coagulation of protoplasm; (3) degeneration through vacuoles or bubbles; (4) swelling of protoplasm.

The appendix deals with (1) the change in protoplasmal structure produced by stains and other chemicals; (2) the precipitation of tannin and other precipitates in the cell sap; and (3) the importation of foreign substances into the living protoplasts. Constant references to the very extensive bibliography are an addition to the usefulness of the book. It would undoubtedly gain in value, especially for the beginner, if it were more amply illustrated.—A. C. Noé.

Mushrooms and toadstools

A recent volume by Güssow and Odell[6] will enable the collector and student of fleshy fungi to add considerably to his understanding of the fungi which he is likely to meet in Canada, and to some extent, in the northern United States. The volume abounds in excellent plates, which are supplemented by brief descriptions. The introductory chapters include a discussion of the nature, structure, and classification of fungi, and a guide to the genera discussed.—G. K. K. Link.

Fungi of Middle Europe

Numbers 5, 6, 7, and 8 of volume I of *Die Pilze Mitteleuropas*[7] have appeared, and continue the excellence of the earlier numbers. Number 5 carries two colored plates devoted to *Boletus regius*, *B. appendiculatus*, and *B. aestivalis*, as well as a plate in black and white devoted to other *Boleti*. The text is devoted to a discussion of *B. regius*. Number 6 carries two colored plates and text devoted to *B. luridus*. Number 7 carries two colored plates, one devoted to *B. elegans* and the other to *B. viscidus*, and a plate in black and white devoted to various *Boleti*. The text is devoted to *B. flavus* and *B. viscidus*. Number 8 carries two colored plates and text devoted to *B. luteus* and *B. variegatus*. It is unfortunate that a work of similar nature is not under way for North American fungi.—G. K. K. Link.

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